The evolution of group traits: modelling natural selection on trait prevalence within and between groups

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Abstract

One of evolution's greatest innovations was group living; indeed, it is fundamental to our daily lives as humans. Yet despite intense theoretical and empirical work, the details of how group living arose and is maintained are poorly understood. A central question in this area concerns the strength of natural selection operating between groups of organisms (group selection) because some think this is key to the evolution of group behaviour. It is, however, challenging to measure natural selection occurring between groups and between the individuals within those groups simultaneously. Consequently, a number of contentious theoretical issues have plagued group selection research for a number of decades, and empirical work on this topic is often misinterpreted. In this thesis, I investigate three biological systems that are candidates for group selection where empirical data is readily available. Using techniques from theoretical and computational biology – simulations, game theory and population genetics – I model evolution occurring at multiple levels simultaneously (multi-level selection), shedding light on the evolution and maintenance of group traits. First, I consider the evolution of a trait – lateralization – at the population- and colony-level in eusocial organisms, which have a reproductive structure that promotes group organisation and cooperation. I provide an evolutionary explanation for the strength of lateralization in colonies of the red wood ant, *Formica rufa*, as a compromise between intraspecific and predatory interactions. After extending the analysis to involve predators targeting multiple colonies simultaneously, I show that populations should tend towards an equal distribution of left- and right- lateralized colonies, resulting in zero population-level lateralization. This contradicts the established view that sociality should produce strong levels of lateralization at the population level. Second, I study a sub-social spider, *Anelosimus studiosus*, which is a group-living species that has recently been claimed to exhibit group-level adaptation. I use evolutionary game theory to explain the evolution of colony aggression with individual costs and benefits, providing an alternative to the existing group-level interpretation. The model generates a striking fit to the data without any between-group interactions. Therefore, I conclude that more evidence is needed to infer group-level adaptation in this colonial spider. Third, I study the Solanaceae, a plant family whose breeding system is reported to have undergone species selection – group selection acting on whole species. I investigate the evolution of self-fertilization over the family's phylogenetic history. By integrating an existing phylogeny with models of breeding system evolution at the individual level, I find the average selection pressure – and attendant properties of populations – expected to have characterised the Solanaceae over ~36 million years. In conclusion, I have shown the power of modelling approaches to clarify evolutionary explanations, to question existing interpretations, and to identify experiments that can help researchers identify the true causes of trait evolution.

Declaration

I hereby declare that this thesis has not been, and will not be, submitted in whole or in part to another University for the award of any other degree.

Signature: .....

Dedication

To Ros, Mum, and Dad. This one's for you.

(No, you don't have to read it all.)

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Chapter 1

Introduction

Evolutionary questions that address multiple levels of the biological hierarchy offer a particular challenge to researchers. There is lack of consensus among biologists about the level(s) at which Darwinian natural selection should be considered to act (Okasha, 2006; Wilson and Wilson, 2007; Wilson, 2008; West et al., 2008). Are traits evolved primarily in the interests of individuals or do some traits evolve in part for the benefit of groups or whole species? It is essential to determine in whose interests natural selection is operating to provide an accurate causal account of evolution (Okasha, 2015), and predict future observations. This is especially important when considering the evolution of social behaviour, in which an organism's actions affect not just their own evolutionary fate, but that of others in their species or group as well. This area of inquiry, termed the *levels of selection*, has a complex history. Many of the fundamental issues date from the 1960s and 1970s: the group selection controversy, theories of multi-level selection and kin selection, and the gene-centred view of evolution, yet it has seen a recent resurgence of interest, both theoretical and empirical.

The renewed interest in these problems promises to advance our understanding of social evolution, though semantic debates have arguably held the field back. Nevertheless, many questions remain unanswered: *pragmatic* questions about how evolution should most productively be modelled, and *empirical* questions concerning particular organisms and their characteristics, which focus upon whether natural selection between groups has been a contributing force in their evolution. The aim of this thesis is to contribute to answering such empirical questions using methods from theoretical biology.

In the next section I outline the background to the group selection controversy and summarise the contemporary debates. I then introduce the tradition of mathematical and computer-based modelling techniques in evolutionary biology, and their potential to advance the field's understanding. Finally, I outline the particular bio-

logical systems investigated in this thesis, constituting problems of significant independent interest, while also directly addressing current issues in the group selection debate.

1.1 The group selection controversy

There are three foundational principles to Darwin and Wallace's (1858) theory of natural selection (Darwin, 1859): (1) Variation: organisms in a population vary in their characteristics. (2) Differential success: some of these characteristics cause organisms to reproduce more often than others. (3) Inheritance: these characteristics may be passed down to an organism's offspring. As a direct result, organisms will tend to become more adapted to their environment over time. In its full generality, the theory applies to anything with variation, differential success, and inheritance (Price, 1970; Lewontin, 1970; Maynard Smith, 1987). The mechanism is not limited to any particular type of entity or system; its logic operates anywhere that these three criteria are met. However, within biology, the empirical question of which entities or systems undergo natural selection is controversial.

Biological systems can be usefully understood hierarchically: species consist of organisms, consisting of organs and tissues, consisting of cells, containing chromosomes, containing genes. The three criteria for natural selection can be theoretically met by many candidates in the biological hierarchy, but in order to correctly identify the causes of organismic traits and behaviour, researchers need to know which levels are most likely to contribute to evolutionary change. An understanding of the way selection at multiple levels may interact with one another, their relative degrees of importance in predicting evolutionary outcomes, and whether any levels can be considered subsumed by any other, are central points of contention. The answer to these questions determine the approach theoretical and empirical biologists should take when investigating natural evolution, especially in the case of social behaviour, where organisms interact with one another in ways that affect their propensity to survive and reproduce (their *fitness*).

The result of natural selection is *adaptation*. It modifies the way biological systems work over time, as more appropriate or adaptive traits promote the survival and reproduction of their carriers. Many traits are intuitively understood to be individual-level adaptations; organisms appear to be adapted – often very precisely – to their environment. However, some traits do not seem to make immediate evolutionary sense within this framework. Altruistic behaviour is an important example, and has been central to the ongoing debate over the levels of selection. Altruists should not be favoured by evolution because they donate resources to others

at a cost to themselves. This should reduce the fitness of altruists relative to those non-altruists receiving their donations, and over time, extinguish altruists from the population. In contrast to this simple evolutionary hypothesis, altruistic behaviour appears to be prevalent in nature. This contradiction provoked the initial inquiry into the levels of selection beyond that of the individual, and altruism remains, to this day, the most commonly considered class of problem when exploring the levels of selection in evolutionary biology.

1.1.1 The good of the group

Darwin (1871) originally suggested that altruism may have evolved by selection at a higher level than the individual: the tribe. A tribe with a higher proportion of altruists, Darwin suggested, may have a competitive advantage over a group with a lower proportion, allowing cooperative groups to outgrow selfish groups. Altruism, therefore, would evolve "for the good of the group". This immediately posits a conflict of interest between levels: individuals benefit from selfishness, but the group they are in benefits from altruism. For *group selection* of this kind to explain the evolution of an altruistic trait, the group selection process must be stronger than the individual selection process for the trait.

Historically, little attention was paid to this conflict of interest however; adaptations were assumed to in some cases evolve to benefit the species or group, without any particular decomposition (Lorenz, 2002; Allee et al., 1949; Okasha, 2006). Wynne-Edwards (1962) was among the first to propose an explicit group selection process, but group selection as a whole was shortly to be brought into question by the work of some other prominent evolutionary biologists, whose *gene-centred* approach was derived from earlier work in *population genetics* and the *modern evolutionary synthesis* (Huxley, 1942).

1.1.2 Population genetics and gene-centrism

In the 1930s, Fisher (1930), Wright (1931) and Haldane (1932, 1955) started to integrate Mendel's (1866) laws of inheritance with Darwinian natural selection. Mendel's (1866) pea plant experiments, from which his laws were derived, are now famous, but their scientific importance was not appreciated until 1900 – thirty-four years after their original publication, and sixteen years after his death (Bowler, 2003). Mendel's (1866) work provided evidence that the traits of organisms were inherited not through a continuous blending of parental characteristics but through a collection of discrete units: genes. Using this new understanding of inheritance, Fisher (1930), Wright (1931) and Haldane (1932, 1955) built mathematical models

to predict how genes would spread throughout populations under different conditions. These three authors worked largely independently, and had certain points of disagreement (Bowler, 2003). Nevertheless, their general approach, termed *population genetics*, became extremely influential after its integration into mainstream biology, culminating in the modern evolutionary synthesis (Huxley, 1942; Bowler, 2003). It laid the foundation for a view of evolution that started to privilege the gene as the base unit of adaptation. From this perspective, the effects of evolution could be reduced down to the changing of gene frequencies in a population (Haldane, 1955; Williams, 1966; Price, 1970; Dawkins, 1976; Okasha, 2006).

Following population genetics, the theoretical work of Hamilton (1963, 1964a,b) and Williams (1966) came to form a new perspective of evolution that was termed the *gene-centred view*. The gene-centred view considers evolutionary adaptation from the perspective of the genes, fully embracing the population genetics notion that what ultimately matters is the proportion of competing genes in a population over time. Individual organisms could then be considered as mere temporary collections or carriers of genes. An adaptation should evolve if it improves the survival or replication of the gene itself – whether it is in the interest of any particular individual that is carrying the gene or not (Hamilton, 1963). Of course, many or even most adaptations will be in the interest of the carrier organism, because the fate of the individual and the genes it carries are heavily entwined. The gene-centred view followed from the observation that, ultimately, traits are encoded in genes, and so those that have evolved must have been in the gene's interest for them to have survived at the expense of alternatives. Researchers should, therefore, only consider a given gene's effects on its probability of replication because genes are the final carrier (or unit) of information when it comes to character traits (Dawkins, 1976).

1.1.3 Kin selection and the rejection of group selection

Hamilton's (1963, 1964a,b) new theory for the evolution of altruism was the cornerstone of gene-centred thinking. He reasoned that altruistic behaviour could be selected for by evolution if the benefit were conferred to relatives of the individual, because those relatives are more likely to also have the altruistic trait (or gene), helping promote it in the population at large. Put another way, a gene for performing an action can proliferate by improving the fitness of other individuals that carry the gene, even if the organism performing the action pays a cost for doing so. This mechanism was later termed *kin selection* (Maynard Smith, 1964). As summarised by Okasha (2006, pg. 145), "inducing its host organism to behave altruistically towards relatives is a 'strategy' that a gene can use to boost its representation in future generations".

Hamilton's rule is the core of kin selection theory. It states that for a trait to be selected for by evolution, the benefit of that trait to the recipient, b, multiplied by the degree of relatedness between the recipient and the carrier of the trait, r, must be greater than the cost of having that trait, c:

$$rb > c \tag{1.1}$$

The degree of relatedness, r, is the probability that the recipient shares any given gene with the donor above the baseline probability of a random individual in the population having that gene (Hamilton, 1970, 1972; Grafen, 1984). A value of $r = 0$ implies the recipient is no more likely to share genes with the donor than it is with any individual in the population, while a value of $r = 1$ implies the donor and recipient have identical genetic makeup. The role of relatedness in Hamilton's rule was originally considered as owing only to direct relatedness (Hamilton, 1964a; Marshall, 2015) (family/kin – genes passed down *by descent*), but has since been generalised to sharing genes for any reason (Hamilton, 1970, 1972; Marshall, 2015), e.g. positive assortment, where individuals are more likely to interact with those similar to themselves (Eshel and Cavalli-Sforza, 1982; Pepper and Smuts, 2002). Hamilton's rule works by identifying the conditions under which the cost of the trait is likely to be redeemed by conferring the benefit on organisms who also carry the gene for the trait. Even though a trait may be altruistic at the level of the organism, when considered from the gene's perspective, the action is self-interested. Dawkins's (1976) *The Selfish Gene* extols the virtues of this theoretical approach.

Kin selection theory is also referred to as *inclusive fitness* theory. The inclusive fitness of a phenotype is the total of all fitness effects it exerts on the individual, plus all fitness effects it has on the individual's relatives, weighted by relatedness. Thus, kin selection theory and inclusive fitness theory hold that individuals evolve to maximise their inclusive fitness.

This elegant new proposal for the evolution of altruism prompted closer investigation into group selection processes, casting doubt on whether they were needed to explain altruism, or if they were even feasible in general. The most prominent criticism of group selection came from Maynard Smith (1964, 1982) and Williams (1966), who suggested that group selection could only occur under a limited set of parameters (which may never occur in nature), and would generally be very weak compared to individual selection. Altruistic groups could not be maintained, they argued, because a mutation for selfishness in the group would have significant within-group evolutionary advantage (by taking advantage of the generosity of others). This mutation would then spread very effectively, causing the group to collapse into widespread selfishness. The group selection process would need to

be particularly powerful to overcome the individual incentive to cheat within each group.

The case against group selection only continued to build as more models for the evolution of altruistic behaviour – in the absence of group selection – were developed, based on reciprocal altruism (Trivers, 1971)[1] and evolutionary game theory (Maynard Smith and Price, 1973; Maynard Smith, 1982). Group selection consequently fell out of favour in the 1960s and 1970s because observed evolutionary outcomes could be explained without it, while imposing fewer assumptions. It was still maintained as theoretically possible, but unlikely to be found in nature (Maynard Smith, 1976).

Gene-centrism is not necessarily incompatible with group selection, because selection between groups of the requisite strength could certainly change gene frequencies in the total population. However, proponents of the gene-centred view in the 1960s and 1970s (predominantly Williams (1966) and Dawkins (1976)) tended to privilege kin selection explanations of social behaviour. They actively argued against many group selection claims of the time (which lacked sturdy empirical support), offering explanations that required no between-group selection. Their explanations instead focused on fitness interactions between individuals from a gene-centred perspective. They essentially used kin selection and gene-centrism to *explain away* group selection.

1.1.4 Multi-level selection

Beginning in the 1970s, several authors began to present more formal models of group selection (e.g. Price, 1972; Wilson, 1975). David Sloan Wilson and Elliot Sober, particularly, questioned whether group selection should have been dismissed in its totality, and attempted to breathe life back into the idea (Wilson, 1989; Wilson and Sober, 1994). Echoing Price's (1972) fully general formulation of group selection, Wilson (1989) argued that evolution should be considered to act on multiple hierarchical levels simultaneously, the effects at different levels having more or less impact on a case-by-case basis. He believed evolutionary processes occurred in the same way irrespective of whether the units are genes within individuals, individuals within groups, and so on. He particularly promoted the idea that groups need not be structures with permanence, but could instead just be sets of individuals that interact on occasion during their lifetimes (Wilson, 1975, 1977). He went further and suggested that group selection could be more liberally applied in the

[1]Some argue reciprocal cooperation should not be classified as altruism (e.g. Marshall, 2011b), but I here use a more intuitive definition of altruism (see Glossary, page 105; Trivers, 1971; Wilson, 1992; Wilson and Sober, 1998; Fletcher and Zwick, 2006; Silk, 2013) that could more precisely be understood as *proximate* altruism.

social sciences to shed light on the evolution of institutions, morality, and controversially, religion (Wilson, 1980, 2002). This reformulation of group selection was termed *multi-level selection* (MLS), emphasising the belief that such selection processes could occur on many different levels of the biological hierarchy, not just on individuals and groups (Wilson and Sober, 1994).

Wilson and Sober (1994) also claimed that kin selection and even evolutionary game theory were in fact group selection under new names, arguing, for example, that kin selection was merely group selection acting on groups of related individuals. The close relatedness of these groups, they claimed, enhanced the effect of group selection by creating more between-group variation – because there is more genetic variation between different families of related individuals than within those families. They insisted it was the differential success of groups of kin (and therefore group selection) that explained the evolution of altruistic behaviour, though they did allow that kin selection was an alternative interpretation. They further argued that the existence of groups had been relegated to an element of the individual or gene's environment, but that this was an aesthetic choice, and the same group selection process was still occurring, whether it was considered as merely peripheral to the individual or otherwise (Wilson and Sober, 1994, 1998).

Damuth and Heisler (1988) separated multi-level selection models into two categories, commonly referred to as MLS1 and MLS2. These two very different types of models had previously been conflated and interspersed in arguments (Okasha, 2006). The difference is important, because they offer distinct criteria for identifying group selection. The following descriptions of MLS 1 and 2 (based on Damuth and Heisler (1988) and Okasha (2006)) are given in reference to modelling individuals in groups, though they may in principle apply to any pair of levels in the biological hierarchy.

Multi-level selection type 1

MLS1 gives primacy to the individual: it is an individual characteristic that the modeller is interested in understanding the evolution of, e.g. propensity to give altruistically. The individuals belong to groups (defined by the implications on behaviour, reproduction, and so on for a particular case), but what is ultimately of interest is the frequency of a given individual trait in the total population over time. The fitness and phenotype of a group is, therefore, equal to the average of its members' fitness and phenotypes – the group is not considered an additional entity with properties of its own, at least in the evolutionary analysis. In MLS1 scenarios, the heritability of the characteristic at the group level in particular is not of concern because inheritance occurs from organism to organism, at the individual level. Group selection in MLS1 occurs when groups contribute different numbers

of *individual* offspring to the next generation, due to differences in the distribution of *individual* traits (encoded in genes) among those groups. The earlier altruism example fits the MLS1 framework: groups with a large proportion of altruists may contribute more offspring to the next generation than groups with a larger proportion of selfish individuals.

Multi-level selection type 2

MLS2, on the other hand, considers the group as a distinct unit of evolution, alongside individuals. MLS2 groups must themselves reproduce, giving rise to offspring groups. The modeller therefore considers neither of the two levels as primary in MLS2. There is a population of groups, each with their own group traits and group fitness. An explicit Darwinian process occurs between these groups, with differential rates of survival, extinction or group reproduction. Simultaneously, inside each group, another Darwinian process occurs, this time considering the traits and fitness of individuals within each group, and changing the make-up of each particular group. Certain *species selection* hypotheses fit the MLS2 framework. Species selection is the operation of natural selection between whole species, where properties of species (that are heritable) cause them to have different rates of speciation or extinction (examined in Chapter 4). For example, in the late-Cretaceous period, the geographic range of some species of molluscs appears to have been a heritable, species-level trait, and those mollusc species with greater geographic range went extinct less frequently, growing their representation on the tree of life as a result (Jablonski, 1987). As noted by Okasha (2006), the propensity for a species of mollusc to disperse widely is probably underwritten in organism-level traits, but the differences in species- (or group-) level production of new "offspring" (through speciation and extinction events) is of independent evolutionary interest. We may also be interested in the evolutionary process occurring within each species, but this can be considered orthogonally, unlike in MLS1 models.

1.1.5 Contemporary debates

The reception to multi-level selection and the reintroduction of group selection has been mixed (Okasha, 2006; West et al., 2007, 2008), with Kramer and Meunier (2016) summarising popular opinion thus: "Most biologists prefer kin selection over multilevel selection approaches as a matter of habit or personal taste [(West et al., 2008, 2007)]". The key issues that remain in dispute are outlined by the following three questions:

1. Are kin and group selection equivalent?

Most theorists agree kin selection and multi-level selection are equivalent for most purposes (West et al., 2007; Wilson, 2008; Marshall, 2011a; Okasha, 2015; Kramer and Meunier, 2016). There are some hypothetical models where one or the other approach appears misleading (van Veelen, 2009; Allen et al., 2013; van Veelen et al., 2014), but it is not clear these are of more than theoretical interest (Queller, 2016). Nevertheless, there is an extensive literature on this question, growing by the year (Michod, 1982; Grafen, 1984; Wilson and Sober, 1998; Wilson and Wilson, 2007; Foster, 2009; Queller, 1992; Nowak et al., 2010; van Veelen et al., 2012; Kramer and Meunier, 2016).

2. Which approach is superior?

Although most agree the approaches are formally equivalent, which theory is more useful is far from settled. The late 2000s saw this issue rise to prominence, with West et al.'s (2007, 2008) exchange with Wilson (2008). West et al. (2007, 2008) lauded inclusive fitness theory and argued that group selection theory has offered little but confusion. Wilson (2008), in turn, argued for pluralism – both approaches should be embraced – and emphasised that group selection had offered insights of its own and was a productive framework for social evolution theory. The decade rounded out with a notable article by Nowak et al. (2010) in *Nature*, claiming that kin selection had been of little use to the understanding of eusociality – a highly controversial claim, because kin selection has been the dominant explanation for eusociality for decades, and vast amounts of empirical work on eusociality make direct reference to kin selection or inclusive fitness theory (Nowak et al., 2010).

Eusocial species are highly social animal societies (such as ant colonies) that meet three criteria: some organisms in the society forego reproduction (e.g. sterile workers in ant colonies), there are overlapping generations (e.g. the queen lives alongside adult offspring), and there is cooperative brood care (Wilson, 1971). Being paradigmatic examples for the evolution of cooperation and altruism (Ratnieks et al., 2006), eusocial societies have always been a mainstay of the kin selection and group selection debate (Foster et al., 2006; Hughes et al., 2008; Bourke, 2011a; Kramer and Meunier, 2016). Consequently, Nowak et al.'s (2010) article received many prompt replies (Abbot et al., 2011; Boomsma et al., 2011; Strassmann et al., 2011; Ferriere and Michod, 2011; Herre and Wcislo, 2011; Bourke, 2011b; Rousset and Lion, 2011; Marshall, 2011a), one of which undersigned by 137 authors (Abbot et al., 2011), contesting Nowak et al.'s (2010) central claim by citing many instances of the productive use of kin selection in understanding aspects of eusociality. This exchange is just one example of a disagreement over the pragmatic value of kin vs.

multi-level selection; heated debates continue (Nowak et al., 2011; Gardner et al., 2011; Birch and Okasha, 2015; Marshall, 2015; Liao et al., 2015; Queller et al., 2015; Nowak and Allen, 2015; Marshall, 2016).

A novel approach to this question arose recently, employing causal graphs to determine the aptness of each theory to describe particular scenarios (Okasha, 2015). Rather than being concerned with which theory has been or will be the most useful in general, Okasha (2015) presents the notion of an "adequate causal representation" of biological phenomena, attempting to determine when kin selection and multi-level selection provide accurate causal accounts of the underlying biology. He claims that the choice between kin selection and multi-level selection is not aesthetic or a matter of theoretical convenience, but instead is an objective matter: which theory captures the relationship between group characteristics, individual fitness and group fitness without spurious correlations? He further argues that neither approach should be deemed globally superior, after providing a number of plausible scenarios where either multi-level selection or kin selection is not causally adequate.

While this approach appears to be gaining momentum (Marshall, 2015; Krupp, 2016; Kramer and Meunier, 2016), it remains to be seen whether evolutionary biologists on both sides of the group selection debate will agree that such a criterion is useful and objective.

3. When does group selection lead to group adaptation?

Gardner and Grafen (2009) make a distinction between group selection and group adaptation. They compare group adaptation to individual adaptation under natural selection, and argue that for the term adaptation to be correctly applied, evolution should appear to be optimising. That is, individual adaptations are so in virtue of the fact that they are (or tend towards) the optimal characteristics for those individuals. The term group adaptation, then, should only apply when evolution optimises group characteristics to benefit the group.

Gardner and Grafen (2009) claim that group optimisation (and therefore group adaptation) is most likely in clonal groups, where all individuals of the group have the same genes, and in societies where there is suppression of internal competition, such as worker policing in honeybee colonies (where worker bees prevent other workers from giving birth to male offspring so that only the queen is able to (Ratnieks and Visscher, 1989)). However, Okasha and Paternotte (2012) show that a lack of within-group variance in fitness is vital to Gardner and Grafen's (2009) optimisation regime. Zero within-group variance in fitness is ensured by the suppression of internal competition (such as worker policing), but clonal groups do not offer such a guarantee because there may be non-genetic effects on individual fitness. Therefore,

the suppression of internal competition may be the only path to group-level optimisation and group adaptation on Gardner and Grafen's (2009) account (Okasha and Paternotte, 2012).

Some evolutionary biologists think this is too high a bar to place on the term adaptation (Sober and Wilson, 2011; Pruitt and Goodnight, 2015). Pruitt and Goodnight (2015) note that in nature, it is very unlikely that any trait is adapted for just one selection pressure, so demanding that an adaptation be driven exclusively by pressure at the group level seems unreasonable. In a similar vein, Sober and Wilson (2011) take particular issue with Gardner and Grafen's (2009) characterisation that "the function of individual-level adaptation is to maximize inclusive fitness and [...] this obtains irrespective of the relative strength of within-group vs. between group selection." This is asymmetric, say Sober and Wilson (2011), because adaptations that are the result of a compromise between individual-level selection and group selection (or even those resulting overwhelmingly from group selection) would still be termed *individual* adaptations, yet elsewhere Gardner and Grafen (2009) insist that the term *group* adaptation only applies to cases where there is zero individual-level selection.

Kramer and Meunier (2016) highlight that, while a notable point of contention in the group selection debate, what counts as a group adaptation is certainly a semantic, rather than an empirical or pragmatic, question.

1.2 Theoretical biology and evolutionary modelling

In this thesis, I address levels of selection questions from a theoretical biology perspective, employing evolutionary game theory and computer simulations. I investigate three biological scenarios that each have a firm empirical grounding, allowing me to focus on the overlap between contemporary theoretical debates and live issues in empirical research. The rationale for this methodology is described below in *Research approach*.

Theoretical methods generally have a long history in evolutionary biology, and while perhaps not as integrated as theoretical and empirical physics (Hillis, 1993; Haller, 2014), biology has seen substantial advances in our understanding owing to evolutionary theory (Huxley, 1942; Hamilton, 1963, 1964a,b; Dawkins, 1976; Servedio et al., 2014).

The timescales for natural evolution are vast and beyond observational study in most taxa[2]. Biologists are left with only a small snapshot of life with which to build theories about the evolutionary past, and the availability of empirical data is

in many cases insufficient to answer biological questions conclusively (Johnson and Omland, 2004; Turchin, 2013). The group selection debate has remained contentious in no small part due to this inability to assess each case cleanly and empirically. Ideally, the field proceeds as an interplay between theoretical and empirical work. In practice, there is more separation between these approaches, and their researchers, than desired (Haller, 2014).

Empirical work involves the recording of population data for particular species, observation of their characteristics and behaviour, and the variation of these characteristics by species, geographical location, and so on. Experimental approaches may actively manipulate aspects of an organism's environment to test how they respond, giving insight into the behavioural mechanisms that have evolved. Laboratory testing of individual and collective behaviour serves the same function, allowing more controlled and accurate measurement, but at the cost of introducing error if organisms react considerably differently than in their natural environment. DNA can also be sequenced from organisms directly, allowing researchers to document how genes are statistically distributed amongst individuals within populations, as well as over space and the ancestral tree of life. *Molecular phylogenies* may be employed to infer the relationships between many different branches in the tree of life, by analysing molecular differences in DNA sequences and how these differences suggest evolutionary divergence. Such a phylogeny (Goldberg et al., 2010) for the Solanaceae plant family informs the work in Chapter 4.

Theoretical work, on the other hand, proposes theories for how certain adaptations could be evolutionarily beneficial, and provides models to assess how probable certain evolutionary outcomes are compared to others. Models usually involve making many simplifying assumptions, so the results derived should be framed conservatively, ready to be tested to see if they make meaningful (and correct) predictions about the real world. A theory is scientifically useful in as much as it is explanatory, and makes successful predictions (Rutherford and Ahlgren, 1991). A theoretical model, then, can have mathematical rigour and explanatory elegance, but its true test is when it is applied to the biology. Good models help biologists understand particular biological facts more clearly, and make predictions about features of the natural world.

It is also well established that scientific theories should be falsifiable (Popper, 1963). Popper (1993) himself expressed some misgivings about evolutionary theory, and specifically whether "survival of the fittest" was falsifiable. He later recanted (Popper, 1978), realising that what he originally viewed as a tautology – those

[2]Exceptions to the rule include bacterial evolution (Blount et al., 2008) and *Drosophila* experiments (Burke and Rose, 2009), where very short generation lengths allow for the study of evolution within human lifetimes.

organisms that survive are those that must have been fit enough to survive – was only a component of the theory of evolution, and that many parts of the theory are in fact falsifiable. Maynard Smith (1982) further notes that particular hypotheses generated within evolutionary theory, including those stated as theoretical models, make assumptions about causal mechanisms that can be tested empirically.

Pure mathematical models have been used extensively, and indeed feature in chapters 2 and 3, but they cannot capture the complexity of interactions in all cases. *Individual-based modelling* techniques can be used to approach problems of this nature, using computer simulations of interacting systems at multiple levels. These models can expose outcomes of theoretical positions that may not be readily apparent (Di Paolo et al., 2000). Their flexibility and speed (relative to empirical work) additionally allow systematic exploration of parameter spaces, testing the robustness and plausibility of proposed ideas.

Evolutionary modelling of this kind is of course a numerical rather than an analytical method of investigation, and is highly stochastic, given the central role random variation and probabilistic selection plays. These constraints come with their own set of complications: all outcomes must be statistical; observation of every individual run is infeasible, making the intuitive understanding of the evolutionary process more difficult; and outcomes are unlikely to bear out elegant mathematical relationships. These costs are significantly outweighed by the advantages: the aforementioned ability to automate parameter sweeps and compute robustness; the freedom to continue to complicate models in desired ways without considering analytic tractability; and in general the ability to work with models of much higher complexity, allowing multi-level relationships to be explored.

1.3 Research approach

There are many interesting philosophical and pragmatic issues at stake in the group selection controversy. However, most theorising on this topic focuses on abstract models aiming for maximally general applicability; it is a field of thought experiments. While certainly productive and helpful in many cases, I argue that a focus on this type of model risks – to invert an idiom – missing the trees for the forest: details are important. Detail-oriented approaches can reveal the actual disagreements by manifesting them in concrete examples. Applying theory to particular biological systems also encourages empirical relevance, contributing to problems that experimental biologists face, and opening new avenues for research that are practicable. I therefore consider three biological systems, advancing current theory to enable a more accurate understanding of the different levels of selection at work.

First, we turn to a complex multi-level scenario involving eusocial insect colonies. Colonies of the red wood ant, *Formica rufa*, were recently observed to exhibit a preference for a particular forelimb (left or right) when crossing a gap in the substrate in which they were walking. Not only were these ants right- or left-biased (*lateralized*) in this task, but in addition, different colonies had different distributions of right- and left-biased individuals within them (Appendix A). Such *colony-level lateralization* is a novel phenomena, lacking an evolutionary explanation. I provide an evolutionary account of this behaviour and for lateralization in eusocial insects more generally. I further suggest a reinterpretation of some recent findings in the growing literature on invertebrate lateralization.

Our second biological system is the highly controversial case of apparent group selection in *Anelosimus studiosus*, a species of social spider that lives in cooperative webs of up to 140 females. Intense debate (Smallegange and Egas, 2015; Grinsted et al., 2015; Gardner, 2015; Pruitt and Goodnight, 2015; Krupp, 2016; Biernaskie and Foster, 2016) has ensued since the publication of Pruitt and Goodnight's (2014) study, which claimed to show evidence of group selection and group adaptation in wild populations of this spider. I develop a model of the system based on well-established theory that provides a striking fit with the data, without a role for group selection. This therefore questions the strength of Pruitt and Goodnight's (2014) evidence for group adaptation; it is further suggested how the dispute could be settled empirically.

Finally, we turn to a form of group selection that has been somewhat neglected until recently: species selection, in which whole species enter Darwinian competition with one another through differential rates of speciation and extinction. Investigating the evolution of the breeding system in the Solanaceae plant family, I build a model that incorporates natural selection at the species level (*between* species) and the individual level (*within* species) simultaneously, elucidating the relationship between these two levels of selection.

1.4 Summary of contributions

This thesis makes a number of original contributions to evolutionary and theoretical biology, summarised below.

- The development of a novel model for the evolution of lateralization in populations of eusocial colonies, incorporating colony coordination, predation and competition. For the first time, lateralization is considered not just at the individual and population level, but also at the colony level. A detailed analysis reveals the extent of colony-level and population-level lateralization that should

be expected under many different conditions. When predators/competitors interact with multiple colonies, the predictions contradict prevailing theory. New experiments are suggested that may distinguish these competing hypotheses and identify the real causes of lateralization above the individual level.

- A first-principles model for success rates in experiential learning of binary characteristics by predators/competitors, formalised by the binomial distribution. Success rates are found to be lowest relative to an ideal predictor at intermediate levels of consistency/conformity (here exemplified by colony lateralization). Incorporated into the above model for the evolution of lateralization, this provides an evolutionary explanation for something previously incompatible with the field's understanding of lateralization: the empirical finding that *Formica rufa* is approximately 60% lateralized at the colony level, but not all colonies are lateralized in the same direction (Appendix A).

- An extension to the Hawk-Dove evolutionary game that considers the effects of variable resource levels and colony size on aggression in a sub-social spider species, *Anelosimus studiosus*, which engages in cooperative capture of prey. This novel combination of evolutionary game theory with empirical data available on colonial spiders provides a compelling, biological explanation for patterns of colony aggression in this species that follows from individual incentives alone, without any between-group competition. Pruitt and Goodnight's (2014) claim of demonstrating group adaptation in *A. studiosus* is refuted by the presentation of this alternative explanation, with the proper attribution of evolutionary causes requiring further evidence.

- The development of a model that combines population genetics (individual-level evolution) and a birth-death phylogenetic model (species-level evolution) to investigate the evolution of self-incompatibility and self-compatibility in the Solanaceae plant family at multiple hierarchical levels simultaneously. This constitutes the first use of such a model to investigate a particular biological system, and is able to provide insight because data is available for this plant family on both population-level factors (such as mutation rate and effective population size) and species-level factors (such as speciation and extinction rates).

- A demonstration that selection on self-compatibility in the Solanaceae is very likely to have been, on average, nearly neutral at the individual level over the ~36 million years of evolution captured by Goldberg et al.'s (2010) phylogenetic data. The model further demonstrates that it is this fact in particular that

allowed species selection to exert its evolutionary influence: maintaining self-incompatibility in this plant family through its lowering of species extinction rates. Species selection is an understudied component of multi-level selection theory, where an evolutionary process occurs at even higher levels than traditional group selection scenarios. The combined model, applied to the Solanaceae, elucidates the relationship between higher and lower levels of natural selection where groups (species) do not merely represent an aggregation of the individuals they contain.

Chapter 2

Colony-level lateralization in a eusocial insect

This work was presented at the *International Society for Behavioral Ecology*, Exeter, U.K., 2016, for an invited talk at the Lateralization Symposium. It is currently being prepared for submission to *PLoS Computational Biology*.

Abstract

Lateralization is the localisation or preference of function on one side of an organism, such as the human tendency to have a dominant left or right hand. If the direction of individual lateralization is distributed randomly within populations (e.g. a 1:1 ratio of right- and left-handers), there is exclusively individual-level lateralization. A population that deviates from a 1:1 ratio of left-lateralized to right-lateralized individuals is said to exhibit population-level lateralization, such as humans' ~9:1 right:left-handed ratio. Population-level lateralization is very common in vertebrates, and some invertebrate species have recently been identified to exhibit such biases as well. However, groups or subpopulations (such as colonies) of any species have not previously been shown to possess directional biases of their own, distinct from other conspecific groups. Yet the red wood ant, *Formica rufa*, has now been shown to exhibit such 'colony-level' lateralization in forelimb preference during a gap crossing task. Of the four colonies tested, one colony has more workers with a bias towards the left forelimb, the other three colonies having more workers with a right forelimb bias. Colony-level lateralization is not predicted by established evolutionary theories of population-level lateralization. We offer an evolutionary account of colony-level lateralization in eusocial insects by considering interactions among workers within colonies, between colonies, and between workers and their predators/competitors. Our model predicts the degree of lateralization seen in wood ant colonies by trading off maladaptive predictability to predators/competitors against adaptive predictability

to colony-mates. We consider the impact of this model on existing empirical work on population-level lateralization in eusocial insects, and reveal the conditions under which colony- and population-level lateralization would be expected to occur more generally.

2.1 Introduction

Binary traits, where a characteristic of an organism can be treated as a dichotomy, are particularly well suited to questions about the levels of selection because they simplify evolutionary analysis. Are organisms bold or shy, solitary or gregarious, right-handed or left-handed? In many cases, traits of interest like these can be usefully categorised on two sides of a boundary, or at two ends of a spectrum, despite being controlled by numerous genes that express many possible points on a continuum (e.g. Maddison et al., 2007; Goldberg et al., 2010; Pruitt and Riechert, 2009). In this chapter, we focus on one example of a binary trait, lateralization, and reveal how close consideration of the levels of selection on this trait can change and even reverse the expected outcome of natural selection.

Lateralization is the localisation or preference of function on the right or left side. The instance of lateralization considered here is forelimb preference: the tendency for an organism to repeatedly use one forelimb rather than another for a particular task. Human handedness provides a familiar example of such a preference: almost all humans have a dominant hand (Hardyck and Petrinovich, 1977; McManus, 2004). Handedness was originally thought to be a trait that separated humans from other animals, but we now know many non-human animal species, including some invertebrates such as locusts and ants, exhibit handedness in a number of contexts (Rogers et al., 2013; Bell and Niven, 2014; Frasnelli et al., 2012b).

Lateralization can occur at different levels. For instance, the vast majority of individual humans have a consistent preference for one hand over the other. If human populations had a random distribution of left- and right-handers, this would be an instance of exclusively individual-level lateralization. However, human populations tend to have many more right-handers than left-handers: approximately 90% of individuals are right-handed (Hardyck and Petrinovich, 1977). When individual lateralization is distributed non-randomly within populations in this way, there is said to be population-level lateralization (Denenberg, 1981; Ghirlanda and Vallortigara, 2004). Population-level lateralization appears to be very common in vertebrates (Rogers et al., 2013), and some invertebrate species also exhibit population-level biases (Anfora et al., 2010; Frasnelli et al., 2011, 2012b).

In addition to individual- and population-level lateralization, a new category has now been discovered in the red wood ant, *Formica rufa*: colony-level lateralization.

Colony-level lateralization occurs when different colonies in the same population are lateralized in different directions (Appendix A). Of four *Formica rufa* colonies tested for lateralized motor behaviour (in this case, gap crossing), three were found to be right-biased and one left-biased. Until now, groups or subpopulations (such as colonies) of any taxa had not been identified as having biases of their own, distinct from other groups in the same species. Group-level lateralization has not been comprehensively studied, but where it has been looked for, it was not previously found. For example, multiple colonies of chimpanzees were observed to exhibit the same right-handed bias, suggesting population-level lateralization (Hopkins et al., 2004). Humans, too, exhibit their strong right-handed bias in all populations that have been studied (Hardyck and Petrinovich, 1977; McManus, 2004). This makes the discovery of colony-level lateralization in *Formica rufa* particularly striking. This novel form of lateralization, while mild in strength, was present in all four of the colonies studied. It was not previously predicted by theory, and lacks an evolutionary explanation.

Both individual- and population-level lateralization have been the subject of much empirical work (Rogers, 2014; Cashmore et al., 2008). The evolution of individual-level lateralization has also garnered corresponding interest in theoretical biology (e.g. Marshall, 1973; Levy, 1977; Frost, 1980; Rogers, 2000; Josse and Tzourio-Mazoyer, 2004). However, there are relatively few works of theory on lateralization above the individual level: one family of models has been particularly influential (Ghirlanda and Vallortigara, 2004; Vallortigara and Rogers, 2005; Ghirlanda et al., 2009). Ghirlanda and Vallortigara (2004) first observe that current theories for the evolution of individual lateralization do not themselves predict any population-level lateralization. They go on to build a model that incorporates the impacts of predation and cooperative social interactions between individuals, and find that the importance of social interactions may predict whether populations are lateralized, and by how much. They find that species with more social interactions should be more strongly lateralized at the population level. However, their model is built with vertebrates in mind, and may not straightforwardly apply to invertebrates, especially species of eusocial insects, whose reproductive habits force us to think differently about inheritance and adaptation (detailed below in Section 2.1.2, and reviewed in Gardner and Grafen, 2009; Hölldobler and Wilson, 2009). An elaboration of their model considers intra-species rather than inter-species interactions (Ghirlanda et al., 2009), with conceptually similar results, but again not accounting for eusociality.

Interest in invertebrate lateralization is growing year on year (Frasnelli et al., 2012b; Frasnelli, 2013; Frasnelli et al., 2012a; Bell and Niven, 2014). Invertebrates make excellent model organisms for the investigation of the evolutionary origins

and maintenance of lateralization due to their simpler nervous systems and experimental tractability (Frasnelli et al., 2014). The prediction of Ghirlanda and Vallortigara (2004), that more social species should be more strongly lateralized at the population-level, has motivated empirical work on invertebrate lateralization. By studying eusocial insect species that have recently diverged from their solitary or sub-social counterparts, researchers can compare the degree of individual- and population-level lateralization present in each, providing a reasonably direct test of the hypothesis (Anfora et al., 2010; Frasnelli et al., 2014).

However, while the logic of the sociality-lateralization hypothesis seems compelling, it is important to note that the model was constructed for vertebrates with only an informal notion of sociality. The model refers to "social" actions as coordinated behaviours that are not necessarily altruistic in nature, performed by organisms that reproduce sexually, with no reproductive division of labour. These interactions are social, but not eusocial. In the context of evolutionary theory, this distinction is fundamental. Eusocial species, such as ants, honeybees and termites, reproduce differently to other species (see Section 2.1.2), and these different modes of reproduction can substantially alter the response to evolutionary selection pressures (Gardner and Grafen, 2009).

In this chapter, we develop a model for the evolution of lateralization at the level of colonies and populations in eusocial insects. We consider the effects of colony-level lateralization on within-colony coordination, and on interactions with predators and competitors, identifying colony phenotypes that produce the highest fitness. We then consider frequency-dependent effects at the population level, determining which conditions predict population-level lateralization.

2.1.1 Related work

Ghirlanda and Vallortigara (2004) provide the first game theoretic analysis of population-level lateralization in their influential 2004 paper. They show that some intermediate degree of population-level lateralization – neither completely homogeneous nor completely random – can be favoured by natural selection. This intermediate level is the result of a trade-off between two opposing forces. On the one hand, there may be positive frequency-dependent selection for individuals to share the majority-side lateralization because being in the minority may be conspicuous when under attack, thereby increasing vulnerability. Shoaling fish provide an illustrative example where miscalibrated movements during predator evasion can be life-threatening: when under attack, a fish that turns left while the majority of its peers turn right would be a much easier target. If group coordination behaviour like this was evolutionarily important, selection pressure would push a population towards homogeneity (strong

population-level lateralization). Conversely, if predators optimise for attacking the prey type they encounter most frequently, those of majority lateralization may be easier to attack as individuals. This would result in *negative* frequency-dependent selection, pushing the population *away* from homogeneity. When opposing instances of frequency-dependent selection are operating, evolution should find a population-level equilibrium, determined by the relative magnitudes of the costs and benefits involved (Ghirlanda and Vallortigara, 2004; Maynard Smith, 1982). Ghirlanda and Vallortigara (2004) consider a coefficient c that defines the relative importance of group versus individual predation effects, and show that intermediate, stable equilibria – between full homogeneity and fully random lateralization – can be produced for a range of values of c.

Ghirlanda and Vallortigara (2004) base their model of group predation – the shoaling fish example – on the *dilution effect* (Bertram, 1978). That is, as more organisms employ the same evasion strategy (left or right), their risk is *diluted*, as they are one of many individuals using that escape strategy, rather than one of a few using the alternative. While this may apply to flock and herd animals, its relevance for invertebrates is more questionable. For example, parasitoids are an important cause of mortality in the red wood ant, *Formica rufa* (Achterberg and Durán, 2011), in which colony-level lateralization has been observed (Appendix A). These parasitoids are typically smaller than their prey, and are unlikely to rush a group when attacking, so a dilution effect may not occur. They do, however, often employ heavily stereotyped movements in their attack sequences and some species have been observed to learn from repeated interactions (discussed below, Section 2.1.4). They may therefore optimise their attacks on the majority-type of prey they encounter, just as predators do in the model of Ghirlanda and Vallortigara (2004).

2.1.2 Eusocial adaptation

Eusocial colonies consist of highly related individuals and exhibit minimal internal competition, as predicted by inclusive fitness theory (Hamilton, 1963, 1964a,b; Ratnieks et al., 2006; Hölldobler and Wilson, 2009; Gardner and Grafen, 2009). They are the paradigmatic example of the evolution of cooperation. Colonies often consist largely of workers: individuals who typically do not themselves reproduce, but are fundamental to the ongoing survival efforts of the colony. By contributing to the colony, these workers are improving their inclusive fitness because they are highly related to the colony's queen(s) and reproductive male(s). In some eusocial insects, worker policing, where workers themselves prevent other workers from raising offspring, can further reduce intra-colony conflict (Ratnieks, 1988; Ratnieks et al.,

2006). The fate of each worker's genes predominantly rest on the success of their shared reproductives, not their own reproductive efforts, and the success of their reproductives in the wider population depends on the efficiency of the colony. Consequently, many traits of individual workers and compositional traits of the colony itself seem to be adapted largely for the "good of the colony". Some researchers thus see eusocial colonies as adaptive units in themselves, and refer to them as superorganisms (Seeley, 1997, 1989; Hölldobler and Wilson, 2009; Gardner and Grafen, 2009), or as the extended phenotype of the queen (Dawkins, 1982). Conflict does still occur within colonies (Ratnieks et al., 2006), but at least in the case of eusocial Hymenoptera (the insect order to which ants, bees and wasps belong), it is rarely violent (Heinze, 2010). We therefore assume that within-colony conflict on worker lateralization is negligible compared to between-colony conflict, and consider only the optimal colony composition (with respect to lateralization) in our analysis.

2.1.3 Lateralization and coordination in *Formica rufa* and other eusocial insects

Owing to the reproductive structure of eusocial colonies, traits that improve cooperation should be selected for because more efficient colonies should outcompete other colonies in the population. If the alignment of individual lateralization improved cooperation in daily tasks, this could go some way to explaining the colony-level lateralization observed in *Formica rufa* (Appendix A), and may also predict colony-level lateralization in other eusocial insects.

Eusocial insect workers, such as those of *Formica rufa*, undergo innumerable cooperative interactions per day, many of which may benefit from coordinated lateralization. If most workers in a colony preferentially use the same forelimb or turn in the same direction during a given type of exchange, coordinated interactions may be more efficient. While efficiency gains may not be substantial at the individual level, once aggregated over the thousands of pairwise interactions undergone each day by a colony's workers, the effects could become evolutionarily meaningful. One example of coordinated behaviour that may be affected by lateralization is route following. Routes laid down by ants via pheromone trails may be easier to follow by its colony mates if they share the same turning bias; indeed, directional biases have been observed in ant route following and nest site exploration (Heuts et al., 2003; Hunt et al., 2014). Another example is the regurgitative passing of food (trophallaxis) between ants, where greater efficiency may be achieved if the motor responses of an ant's partner are similar to those of the other ants it has performed trophallaxis with previously. A *Formica rufa* colony has actually been observed to exhibit a lateral bias in antennae use during trophallaxis (Frasnelli et al., 2012a). Passing

of nest-making material, too, may see fewer items dropped if the ants pass to and fro in a predictable way. All of these cooperative examples point towards a benefit for stronger lateralization at the colony level. The greater the proportion of colony members with the same directional bias, the more efficient small-scale cooperative interactions are likely to be.

Yet, *Formica rufa* colonies do not have very strong lateralization (Appendix A). In this recent empirical work, ants crossing a gap in the substrate they were walking upon showed individual preferences in forelimb reaches. Some preferred to reach with their left forelimb, others with their right, and still others had no preference. These *Formica rufa* ants were taken from four different colonies in a natural population. All four colonies deviated from the null hypothesis (the binomial expectation of random left and right draws from the colony) with statistical significance (Appendix A.1). From three of the colonies, approximately 60% of forelimb reaches were with the right forelimb, while from the other colony, approximately 60% preferentially used their left forelimb. This is a mild colony-level lateralization – far from the perfect uniformity (100%) suggested by the importance of cooperation. There may, therefore, also be costs to coordination.

2.1.4 Lateralization and competitive/antagonistic interactions

Eusocial insect colonies are subject to a number of inter-specific pressures that are competitive or antagonistic in nature. Many of these involve direct fitness costs, for example: predators kill colony members for food (Brian, 1983), parasites extract resources at the expense of the colony (Schmid-Hempel, 1998), parasitoids lay their eggs in colony members and eventually kill them (Cusumano et al., 2016; Godfray, 1994), and workers compete with other species for access to prey (Brian, 1983). On the other hand, some interactions involve direct fitness benefits: ants "farm" aphids, tending to them and consuming their waste product, honeydew (Ivens, 2014), and eusocial insects in general prey on other invertebrates for protein (Lenoir, 2002). Still other interactions involve more indirect fitness costs, such as with parasitoids or predators that prey on aphids, or competition with other species such as ladybirds that feed on aphids' honeydew, reducing ants' access to this resource (Völkl, 2001; Novgorodova, 2015).

Here we will use parasitoids as an example of a fitness-affecting inter-species interaction, because they are estimated to be up to 20% of the insect population of Earth, and are likely to prey on many if not all eusocial insect colonies (Godfray, 1994). Parasitoids reproduce by injecting their eggs into other organisms (here, colony workers); this is termed *ovipositing*. The eggs incubate and then hatch from

within the host organism, draining its resources and ultimately killing it (Godfray, 1994). By being fatal to the individual, attacks by parasitoids can also be a significant problem for colony fitness because colonies rely on the survival and productivity of their workers. Parasitoids often oviposit with highly stereotyped movements (Mowry et al., 1989; Papaj, 1993; Luck et al., 1982; Rogers, 1972), so it is quite possible that small behavioural differences between their targets, like lateralized forelimb preference, may have an effect on success rates. Parasitoids, though not precisely "predators", have an equivalent effect on colony fitness due to the fatality of their attacks (Hassell, 1978), so we will not distinguish between them for the rest of this chapter.

Targeting a worker from its weaker side, or on the side it is less likely to turn, may improve predation (or ovipositing) success. Lateralization in predation is already an established phenomenon; for example, three species of toad were found to be lateralized in predator avoidance (Lippolis et al., 2002). A parasitoid or predator could increase its rate of success by learning, over repeated interactions with many worker prey, which side is more effective for its attacks. Indeed, some parasitoid species have been observed to learn different strategies for ovipositing aphids, over repeated interactions with their guarding ants (*Formica polyctena*, a close relative of *Formica rufa*), and to significantly increase their ovipositing success by doing so (Völkl, 2001). However, parasitoids or predators could only improve their laterality-linked success to the extent that the workers they are targeting are uniform in their lateral bias. If there were no colony-level or population-level laterality bias, then there would be no information to learn from repeated interactions; one worker's reaction would imply nothing about the directional reaction of the next. In the presence of laterality bias, however, predators could learn the most effective side for their attacks over repeated trials. More uniform colonies are more predictable, and may therefore suffer a fitness disadvantage compared to more random rival colonies.

This reasoning assumes parasitoid and predators attack just one colony at a time. They may in fact visit multiple colonies during their lifetimes, or operate in a patch with workers from multiple local colonies. In this case, predictability needs to be considered not just within colonies, but between colonies as well. If most colonies in an area were left-biased, a colony with a right bias may prosper because the predators in the area may learn to be particularly effective against the left-biased majority individuals. This would be an example of negative frequency-dependent selection, where an increase in the frequency of a trait, left-bias, decreases the value of that trait relative to alternatives. In these circumstances, frequency-dependent selection would be operating between whole colonies, at the colony level, instead of at the individual level where it is most commonly considered (Gromko, 1977; Heino et al., 1998).

While we have explained these dynamics in terms of predation and parasitoids, other inter-species interactions mentioned above could also have their costs and benefits affected by lateralization-based predictability, with similar fitness implications. Many of these, for instance, involve one-on-one, interactive agonistic engagements: prey, parasites, competitors for resources, and parasitoids and predators on resource-bearing species like aphids. If such competitors were able to learn the eusocial insects' preferred side for attack or defence, because a colony was strongly lateralized, they could adapt appropriately and colony fitness could suffer as a result. For the remainder of this chapter we will refer to the antagonist as a predator, though in principle the analysis may apply to any of these other categories.

2.2 Materials and methods

Our model assumes a predator attacks workers from the colony it is preying on N_p times. On each of the N_p encounters, it guesses the prey's laterality as left or right, based on whether it has encountered more left- or right-handed prey in the last m_p encounters (its memory capacity), or guesses randomly if there is no majority. We assume the predator is more likely to be successful if it guesses the laterality of its prey correctly than incorrectly. The statistic of interest is therefore the probability of a predator guessing correctly; the relative importance of guessing rightly or wrongly can be determined by coefficients in subsequent calculations of fitness.

The optimal strategy in this binary choice problem is to consistently choose the action that performs best against the most probable outcome (Duda et al., 2012). This has a lower error rate than probability matching, for example, which chooses the best action against each outcome with a probability proportional to that outcome occurring. The optimal, consistent choice strategy is also optimal in Bayesian decision theory (Duda et al., 2012), though a Bayesian approach would compute the expected probability of ants turning right or left in a different way. We take a frequentist approach in this chapter, but consider a Bayesian alternative in Section 2.3.5.

2.2.1 Predator prediction

An individual worker randomly picked from the colony will turn left or right with a probability determined by the colony's laterality bias. We define the colony's laterality bias as a value $-1 \leq x \leq 1$. A bias of $x = 0$ means a random worker is just as likely to turn left as right, while a bias of $x = 1$ means all workers turn right in every encounter, and $x = -1$, that they turn left in each encounter. Note that left and right forelimb preferences are assumed to be symmetric, neither

offering any inherent advantage. Predator prediction then, depends only on the absolute laterality, $0 \leq |x| \leq 1$, which captures the strength of the bias towards the dominant direction (whether it be right or left). We state the remainder of the methods assuming the colony in question is left-dominant ($x < 0$) for concreteness; the results for right-bias are equivalent. All instances of *left* represent whichever direction is dominant, and *right*, whichever direction is in the minority.

The probability of colony workers turning left and right on the ith encounter is independent of the encounter number i, and is as follows:

$$\begin{aligned} \mathrm{P}(\text{turn left} \mid x) &= \tfrac{1}{2}(1 + |x|) \\ \mathrm{P}(\text{turn right} \mid x) &= \tfrac{1}{2}(1 - |x|) \end{aligned}$$

The predator continuously guesses the opposite side to the most common turning direction it has previously observed. Given a memory of the last $i - 1$ encounters, the probability of a predator guessing left on the ith encounter depends on whether more than half of the previous encounters were left turns ($l > \frac{i-1}{2}$). This can be calculated exactly from the cumulative binomial distribution as follows:

$$\mathrm{P}(l > \tfrac{i}{2} \mid i, x) = \sum_{j=\left\lceil \frac{i}{2} \right\rceil}^{i-1} \mathrm{binopmf}(i-1, j, \tfrac{1}{2}(1 + |x|)), \tag{2.1}$$

where $\mathrm{binopmf}(n, k, p)$ is the binomial distribution's probability mass function:

$$\mathrm{binopmf}(n, k, p) = \begin{cases} \binom{n}{k} \cdot p^k (1-p)^{n-k}, & \text{if } k \in \mathbb{N}_0 \\ 0, & \text{otherwise.} \end{cases} \tag{2.2}$$

The predator guesses randomly when the number of left turns observed, l, is exactly half of the number of previous encounters ($l = \frac{i-1}{2}$). The probability of this occurring can be calculated from the binomial distribution:

$$\mathrm{P}(l = \tfrac{i-1}{2} \mid i, x) = \mathrm{binopmf}(i-1, \tfrac{i-1}{2}, \tfrac{1}{2}(1 + |x|)). \tag{2.3}$$

The predator will guess left 100% of the time where $\mathrm{P}(l > \frac{i}{2} \mid i, x)$, and 50% of the time where $\mathrm{P}(l = \frac{i-1}{2} \mid i, x)$. The total probability of guessing left on the ith encounter is therefore:

$$\mathrm{P}(\text{guess left} \mid i, x) = \left(\sum_{j=\left\lceil \frac{i}{2} \right\rceil}^{i-1} \text{binopmf}(i-1, j, \tfrac{1}{2}(1+|x|)) \right) + \frac{1}{2}\,\text{binopmf}(i-1, \tfrac{i-1}{2}, \tfrac{1}{2}(1+|x|)). \tag{2.4}$$

The probability of the predator guessing right is the complement of the probability of it guessing left:

$$\mathrm{P}(\text{guess right} \mid i, x) = 1 - \mathrm{P}(\text{guess left} \mid i, x). \tag{2.5}$$

Therefore, the probability of guessing correctly on the ith encounter,

$$\begin{aligned} \mathrm{P}(\text{success} \mid i, x) &= \mathrm{P}(\text{guess left} \mid i, x) \cdot \mathrm{P}(\text{turn left} \mid i, x) \\ &\quad + \mathrm{P}(\text{guess right} \mid i, x) \cdot \mathrm{P}(\text{turn right} \mid i, x) \end{aligned} \tag{2.6}$$

$$\begin{aligned} &= \mathrm{P}(\text{guess left} \mid i, x) \cdot \tfrac{1}{2}(1+|x|) \\ &\quad + (1 - \mathrm{P}(\text{guess left} \mid i, x)) \cdot \tfrac{1}{2}(1-|x|). \end{aligned} \tag{2.7}$$

The average success rate over a sequence of N_p encounters, with memory capacity m_p, against a colony of laterality x, is denoted by the function $S(N_p, m_p, x)$. Assuming unlimited memory ($m_p = \infty$):

$$S(N_p, \infty, x) = \frac{1}{N_p} \sum_{i=1}^{N_p} \mathrm{P}(\text{success} \mid i, x). \tag{2.8}$$

This is because $\mathrm{P}(\text{success} \mid i, x)$ is defined as the success rate on the ith encounter, based on the predator guessing from its observations of all of the previous $i-1$ encounters. Introducing limited memory, where predators can only use information from the previous m_p encounters, will not affect the first m_p+1 encounters because the memory capacity will not have been exceeded. Consequently,

$$S(N_p, m_p, x) = S(N_p, \infty, x) = \frac{1}{N_p} \sum_{i=1}^{N_p} \mathrm{P}(\text{success} \mid i, x) \quad \text{where} \quad N_p \leq m_p + 1. \tag{2.9}$$

However, where $N_p > m_p+1$, the memory capacity will be limiting for subsequent encounters ($m_p+1 < i \leq N_p$). The success rate for these subsequent encounters will

be equal to P(success $\mid i = m_p + 1, x$). This is because P(success $\mid i, x$) is equivalent to the success rate with a memory of the last $i - 1$ ($=m_p$) encounters. The fully general success rate is therefore:

$$S(N_p, m_p, x) = \frac{1}{N_p} \sum_{i=1}^{N_p} \mathrm{P}(\text{success} \mid i = \min\{i, m_p + 1\}, x). \tag{2.10}$$

2.2.2 Worker cooperation

Worker cooperative encounters are assumed to benefit when workers correctly anticipate the forelimb preference of their cooperators in a similar way to predators, though the magnitude of the evolutionary impact per encounter may be very different. However, unlike predators, they may not have to learn forelimb preference through experience. We assume colony workers are evolutionarily optimised to interact most efficiently with whichever is the majority-type forelimb preference. The success rate of worker cooperative interactions is then equivalent to the probability that a given worker has the majority-type forelimb preference:

$$S_w(x) = \tfrac{1}{2}(1 + |x|). \tag{2.11}$$

If we were to assume instead that workers learn the forelimb preference of their fellow colony mates during their lifetimes, in a similar way to predators, we could redefine the success rate using the previously defined predator S function. Substituting a different number of encounters that workers may undertake during their lifetimes (N_w) and a worker memory capacity (m_w):

$$S_w(N_w, m_w, x) = S(N_w, m_w, x). \tag{2.12}$$

2.2.3 Fitness curves

Whether we use Equation 2.11 or Equation 2.12 to define the worker success rate S_w, we combine it with the success rate of the predator, S, to calculate colony fitness function $F(x)$ in the same manner, for colony laterality x:

$$F(x) = c \cdot S_w(\ldots, x) - S(N_p, m_p, x). \tag{2.13}$$

Coordination success with fellow workers is fitness-positive, while predictability

to predators is fitness-negative. Following Ghirlanda and Vallortigara (2004), the relative importance of cooperation (compared to predation) can be manipulated by a variable c, which we call the coordination coefficient.

2.2.4 Predation on multiple colonies

Colony fitness may also be affected by competition with other colonies in a patch. If predators attack multiple colonies in a local population, they may specialise on the population-level majority direction, potentially allowing colonies that are biased in the opposite direction to benefit. We simulated predation on multiple colonies of varying laterality to determine any population-level effects that may occur between colonies preyed on by common predators.

In order to do this, we calculated the probability of a predator guessing left or right in each encounter, assuming that the predator randomly encountered workers from g (≥ 1) colonies on average. We introduced a new quantity, x_m, the mean laterality of colonies in the population.

Because each worker is drawn independently from the population of colonies, it is mathematically equivalent to drawing from a single colony but with an appropriately adjusted laterality. When we are evaluating the fitness of a focal colony in a population, we know the predator will encounter a fraction $\frac{1}{g}$ of workers from the focal colony (of laterality x) and the rest of the workers, $\frac{g-1}{g}$, from the population at large (of laterality x_m). The adjusted laterality is therefore:

$$A(x, x_m, g) = x\frac{1}{g} + x_m\frac{g-1}{g} = \frac{x + x_m(g-1)}{g} \tag{2.14}$$

The predator is assumed to make its predictions in the same way as if it were preying on a single colony, the only difference is the adjusted laterality. The probability of guessing left and right can therefore be computed as before, by substituting in the adjusted laterality, $x = A(x, x_m, g)$, into Equations (2.4) and (2.5).

However, instead of then computing the prediction success rate of predators according to Equation 2.7, we compare the predators guesses against the probability of the *focal* colony's workers turning left or right. This distinction is important, because it asserts the independence of the predator's general success rate from its success rate against a given colony. For example, the focal colony may be preyed on less effectively at certain values of x, even if the predator maintains a good general success rate. The probability of the predator guessing correctly on the ith encounter is therefore:

$$\begin{aligned}\mathrm{P}(\text{success}' \mid i, x, x_m, g) = \mathrm{P}(\text{guess left} \mid i, x = A(x, x_m, g)) \cdot \tfrac{1}{2}(1 + |x|) \\ +(1 - \mathrm{P}(\text{guess left} \mid i, x = A(x, x_m, g))) \cdot \tfrac{1}{2}(1 - |x|).\end{aligned} \tag{2.15}$$

To calculate the average success rate for mobile predators over N_p encounters with m_p memory capacity, we substitute $\mathrm{P}(\text{success} \mid i, x)$ (Equation 2.7) with $\mathrm{P}(\text{success}' \mid i, x, x_m, g)$ into Equation 2.10:

$$S_{\text{mobile}}(N_p, m_p, x, x_m, g) = \frac{1}{N_p} \sum_{i=1}^{N_p} \mathrm{P}(\text{success}', \min\{i, m_p + 1\}, x, x_m, g). \tag{2.16}$$

To reiterate, the resulting calculation is equivalent to testing the success rate of a predator against a focal colony of laterality x, where it interacts and learns from workers from both the focal colony (with probability $\frac{1}{g}$) and the population at large, with mean laterality x_m.

The fitness of a colony was then calculated using this new success rate that depends on the mobility of the predator g, and the mean population laterality x_m. The predation success rate was again combined with (subtracted from) the coordination advantage multiplied by the coordination coefficient c:

$$F_{\text{mobile}}(x) = c \cdot S_w(x) - S_{\text{mobile}}(N_p, m_p, x, x_m, g). \tag{2.17}$$

x	Laterality bias of the colony. No bias: $x = 0$. Fully biased: $x = \pm 1$.
c	Coordination coefficient (relative importance of coordinated interactions *versus* competitive/predatory interactions)
x_m	Population mean laterality (for predation on multiple colonies)
m_p	Predator memory capacity
N_p	Number of times the predator attacks a worker during its lifetime
m_w	Colony worker memory capacity
N_w	Number of times workers interact with one another during their lifetime
x^{**}	Optimal colony laterality (location of global fitness maxima)

Table 2.1: Variables used in the model of colony fitness and predator/worker learning.

2.3 Results

We built a model of laterally biased eusocial colonies, simulating a predator learning and predicting individual workers' lateralization, to consider how different degrees of laterality may affect colony fitness. We first simulated a predator trying to guess the turning direction of workers encountered from a single colony with a given laterality. The success rate was recorded over different numbers of interactions, and for various memory capacities of the predator. We then considered how workers themselves may coordinate more effectively if a colony was more strongly lateralized, building a model of colony fitness that rewarded colonies both for internal consistency and for being hard for predators to predict. Finally, we considered whether competition within a metapopulation of colonies could change the evolutionary dynamics, by looking at predators targeting patches, and the resulting frequency-dependent selection.

2.3.1 Prediction success rates

When memories are long, the behaviour of a predator can be broken down into two phases: discovery and deployment. In the initial discovery phase, a predator's guesses are likely to be little better than random, as they are informed by limited experience. After some number of encounters, the predator will eventually settle on

predicting that each subsequent worker will turn in the direction most common in the colony, if its memory is sufficient to infer it. We will call the encounters from this point until the end of the sequence the deployment phase, as the predator continues to deploy the same prediction strategy. The deployment phase should have the highest possible success rate, so the overall success rate is strongly affected by what proportion of the encounter sequence is spent in the deployment phase versus the more unreliable discovery phase.

Increasing the colony laterality index x increases the prediction success rate (Figure 2.1a-c). This is because stronger laterality biases shorten the discovery phase, as the majority direction can be inferred with fewer samples from the colony. Starting at $x = 0$, individual lateralization is random, and the prediction success rate is 0.5 for all conditions. At $x = 1$, where every worker from the colony has the same directional preference, the prediction success rate is near 1. More precisely, averaged over many trials, the success rate at $x = 1$ tends to $1 - \frac{0.5}{N_p}$. This is because the first encounter is still a random decision (with a success rate of 0.5), while the remaining $N_p - 1$ encounters are predicted correctly based on this first encounter. Consequently, as N_p increases, the success rate for $x = 1$ tends to 1.

It is worth considering the rest of the results relative to peak prediction, which we define as the expected success rate of an ideal observer as the number of encounters N_p and memory m_p tend to infinity. The peak prediction success rate is equal to $0.5 + \frac{x}{2}$: a linear relationship starting with a success rate of 0.5 when the colony has no laterality bias, to a success rate of 1 when the colony is fully biased with all workers turning the same way. The linear relationship reflects the fact that workers picked at random from a colony can only be correctly guessed to the extent that there is correlation between previous and future experience, i.e. there is some bias in the colony's laterality. When guessing the outcome of an independently random binary decision, the best possible strategy is to discern the more frequent of the two outcomes and to guess that continuously, which is equivalent to our learning model with infinite encounters and memory. This provides a natural upper limit for performance in our simulations. The performance under various conditions may then be compared relative to this peak prediction (Figure 2.1d-f).

Increasing the number of observations N_p tends to increase the success rate, because more guesses are made after the initial discovery phase rather than during (when guesses tend to be less accurate). However, increases in N_p exhibit diminishing returns: the two-fold increase from $N_p = 10$ to $N_p = 20$ increases the success rate by a similar margin to the five-fold increase from $N_p = 20$ to $N_p = 100$. Further, the two-fold increase from $N_p = 100$ to $N_p = 200$ has a much smaller impact on success rates than either of the two previous increases, and is barely visible in the low memory condition ($m_p = 5$, Figure 2.1a,d). Returns are diminishing for increasing

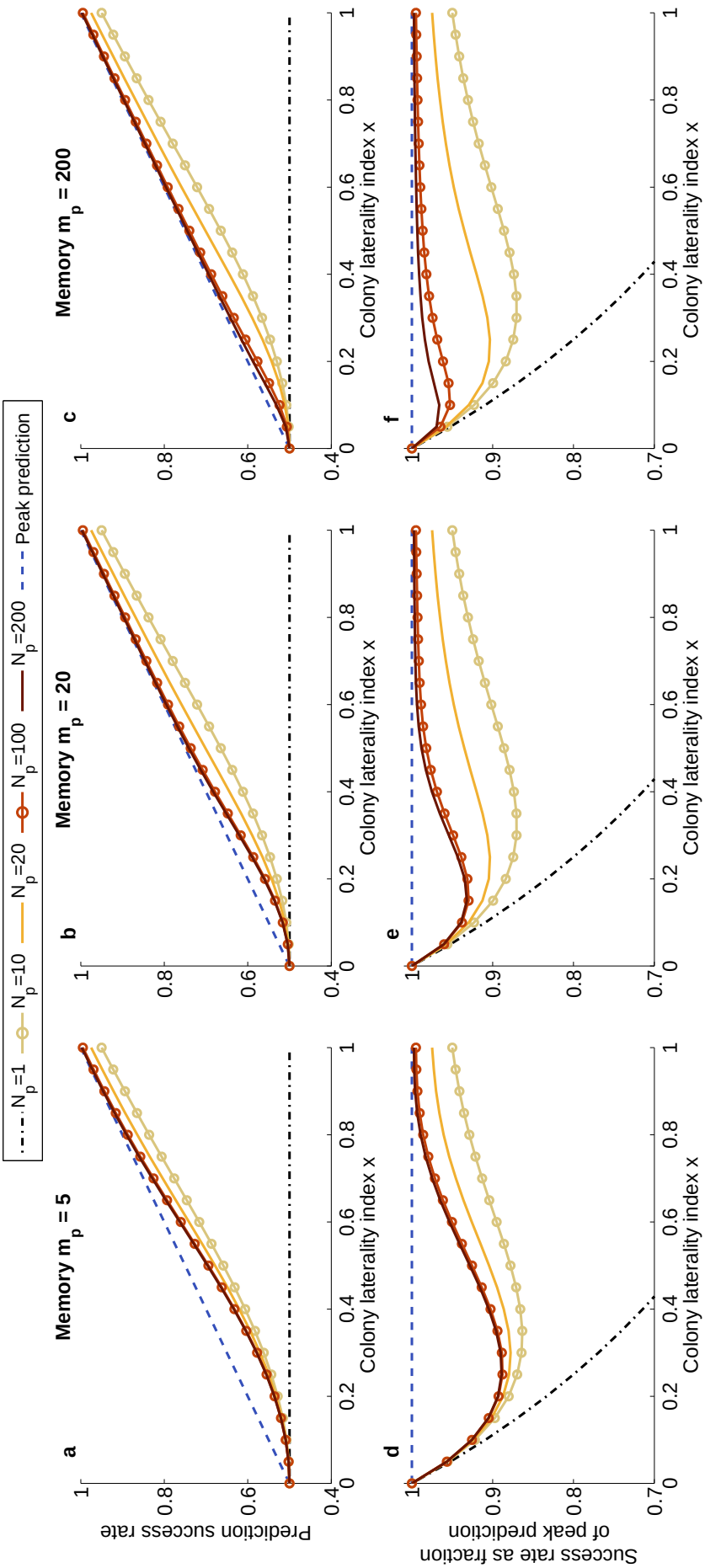

Figure 2.1: (**a-c**) Prediction success for guessing the laterality of workers (binary, left or right) from a colony with a given laterality index. Results shown for simulations with varying memory lengths m_p and number of workers encountered N_p. Peak prediction performance, as N_p and m_p tend to infinity, indicated by the dashed blue line. Baseline performance at $N_p = 1$, where memory cannot be utilised, indicated by the dot-dashed line. (**d-f**) Prediction success as a fraction of peak prediction for each condition.

numbers of encounters because results start to approach peak prediction, where no further improvement can be made.

Success rates relative to peak prediction are generally at their lowest between $x = 0.1$ and $x = 0.4$ (Figure 2.1d-f), with a mild to moderate bias towards one hand rather than the other. Generally, the more coordinated a colony's workers are, the fewer interactions it takes on average to learn the best strategy of attack, and so the closer to peak performance. However, at $x = 0$, the average performance must be the same for all observers (random chance, 0.5), anchoring relative performance at 1. This anchor pushes the weakest *relative* performance to the right, where there is sufficient order for an ideal observer to perform better than chance, but not so much order to allow a predator with limited memory and interactions to quickly infer the best strategy.

Increasing memory m_p increases performance for high values of N_p (≥ 100) by pulling these curves up to peak prediction at lower and lower values of x (Figure 2.1). With longer memories, observers are more able to pick the best strategy over repeated interactions because they have a larger sample size; this is especially pronounced when the signal to noise ratio is limited by low x. This effect also shifts the weakest relative performance to the left for high N_p for the same reason (Figure 2.1d-f). However, at low N_p, where the discovery phase takes up the majority of interactions, the better prediction ability afforded by longer memories cannot be capitalised on, and performance remains approximately equal despite substantial increases in memory size.

2.3.2 Fitness curves

We next considered the outcome of a trade-off between predictability to predators and coordination with fellow workers. We first assumed workers in the colony were optimised to coordinate with the majority directional preference of the colony. Their success rate in coordinated actions was therefore linearly proportional to the laterality of the colony. This is equivalent to peak prediction performance (Figure 2.1a-c, dashed blue lines).

On these assumptions, fitness maxima are produced where predator memory is weakest relative to peak prediction. Due to the shape of the performance curves (Figure 2.1), all conditions produced a single (global) fitness maximum, x^{**}, which identifies the optimal laterality a colony should evolve towards (Figure 2.2). These maxima are generally found at weak to mild laterality strengths ($0.1 < x^{**} < 0.4$).

Biologically, the optimality of mild lateralization (Figure 2.2) is the result of the trade-off between strong lateralization for improved coordination, and weak lateralization for maximally confusing predators. Predator success rates do not

increase with laterality x at the same rate that the coordination benefit does because of the nature of the predator learning model (Figure 2.1), so there are intermediate values of x where the benefits of increased coordination are not outweighed by the detriment of greater predator success rates.

The precise location and magnitude of each fitness maximum is determined by the number of predator encounters and the memory capacity of those predators. As predator memory m_p increases, little change is observed if the number of predator encounters is low, $N_p \leq 40$ (Figure 2.2). This is because additional memory capacity makes little difference at low numbers of encounters (Figure 2.1). However, for higher numbers of predator encounters, $N_p \geq 100$, predator memory m_p is able to make a substantial difference. When predators' memories are longer, their success rate remains closer to peak for lower and lower levels of x (compare Figure 2.1a and c). This is because the lower the strength of colony laterality x, the harder it is for a predator to detect and exploit that laterality bias in predicting the next encounter; a longer memory enables exploitation at lower lateralities. By enabling predators to accurately predict workers' movements at lower lateralities, the weakest performance relative to peak prediction is shifted left, to lower values of x. Therefore, increasing memory reduces the value of x^{**} when there are enough encounters to register the difference ($N_p \geq 100$).

The magnitude of the maximum is principally determined by the number of encounters (Figure 2.2). As encounters N_p decrease, predator prediction becomes weaker relative to peak prediction at most values of x (as the predator has fewer samples to learn from), exaggerating the advantage afforded to colonies that are hard for predators to predict. The magnitude of the fitness peak is also reduced by increased memory, provided that memory is usable in additional encounters (compare e.g. $N_p = 40$ in Figure 2.2a and b; $N_p = 200$ in Figure 2.2b and d). This is because predators make better predictions as their memory capacity increases, reducing the ability for colonies to exploit randomness (weaker lateralization) to improve their fitness.

We next considered the case where, rather than being genetically optimised to coordinate with the majority directional preference, workers learned the preferences of their fellow colony mates during their lifetimes. We employed the same learning process for both workers and their predators, but assumed different numbers of encounters and memory capacities. When workers and predators were assumed to have both the same memory capacity and number of encounters, $N_p = N_w$ and $m_p = m_w$, their success rates cancelled out and fitness was equal for all colony laterality indices (see $N_p = N_w$, Figure 2.3c,f). However, if the number of encounters or the memory capacity of workers was assumed to be higher than that of predators ($N_w > N_p$ or $m_w > m_p$), all conditions produced a single (global) fitness maximum,

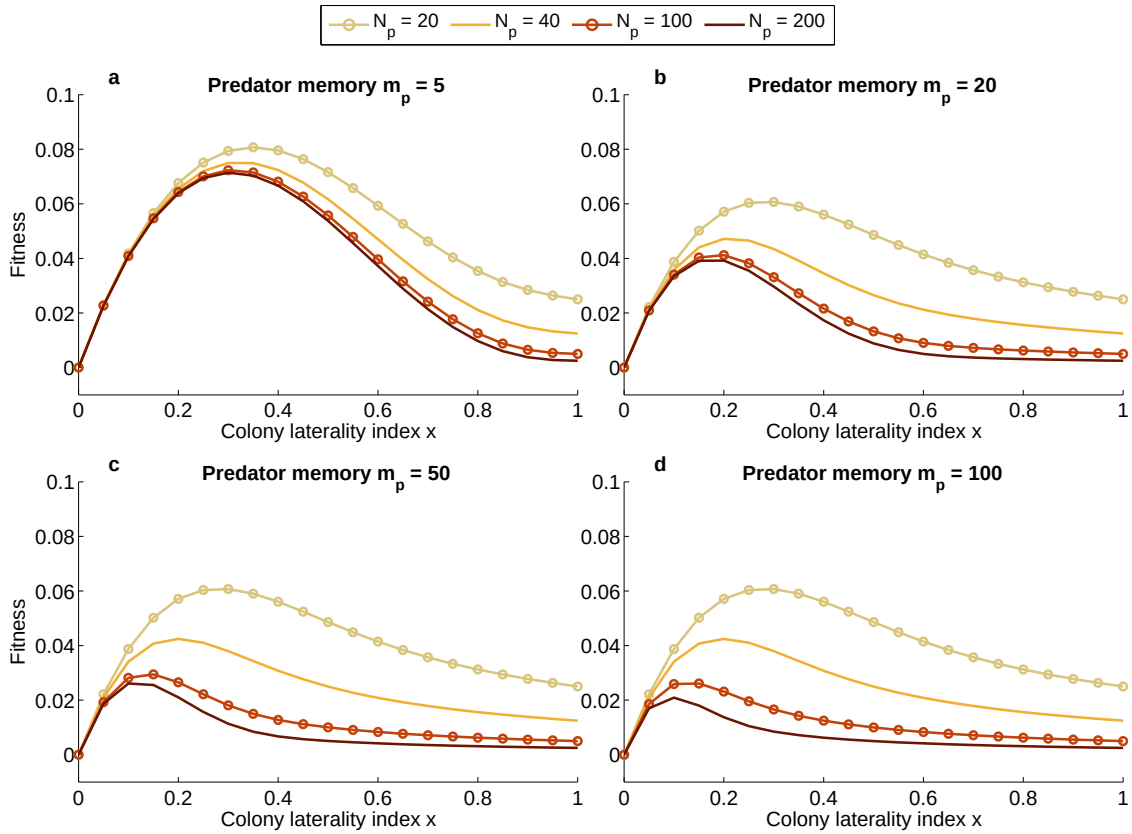

Figure 2.2: The effect of colony laterality index on colony fitness, where predictability to predators is negative but cooperative interactions are aided by increased laterality. Results shown for varying number of predator encounters N_p (line series) and predator memory lengths m_p (columns). Worker coordination success is a linear function of colony lateralization. Coordination coefficient $c = 1$.

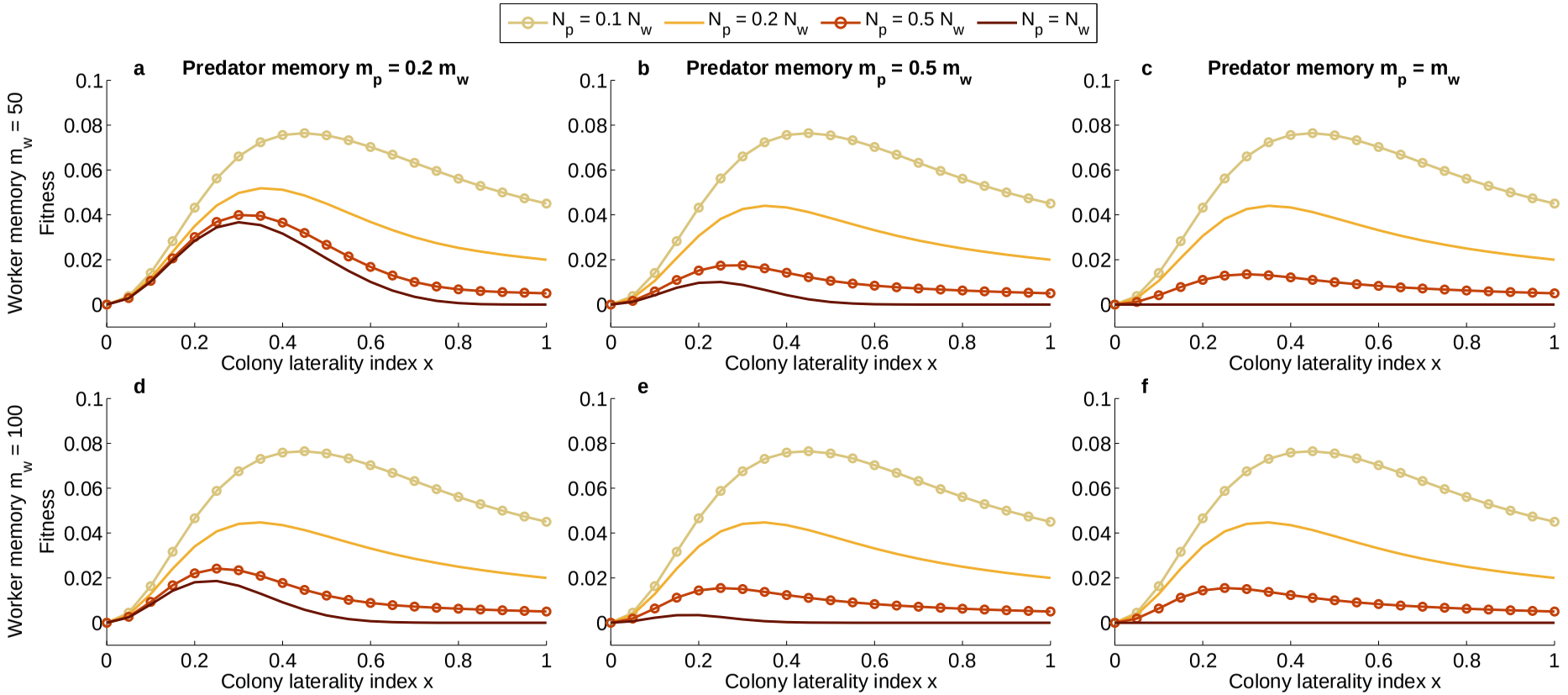

Figure 2.3: The effect of colony laterality index on colony fitness, where predictability to fellow workers is positive and predictability to predators is negative. Results shown for a fixed number of worker encounters $N_w = 100$, and varying predator encounters N_p (as a fraction of N_w), worker memory lengths m_w (rows), and predator memory lengths m_p (columns, as fraction of m_w). Worker coordination success rate is determined by learning. Coordination coefficient $c = 1$.

found at x^{**}, where generally $0.1 < x^{**} < 0.5$ (Figure 2.3). Equivalently, if predators were assumed to have a greater number of encounters or a better memory, a fitness *minimum* was reliably observed between $x = 0.1$ and $x = 0.5$. We will focus on the former case: fitness maxima where $N_w > N_p$ or $m_w > m_p$, which we expect to be more biologically realistic. The previously considered case where colonies are optimised genetically can be seen as an extreme version of this scenario where $N_w \to \infty$ and $m_w \to \infty$ because the colony payoffs for coordination are equivalent (a linearly increasing function of colony lateralization).

The location and magnitude of the fitness maximum is determined by the relative number of encounters and memory capacity of predators when compared with workers. As predator memory m_p increases as a fraction of worker memory m_w, little change is observed if the number of predator encounters is much lower than the number of worker encounters, $N_p \leq 0.2N_w$ (Figure 2.3). This is because additional memory capacity makes little difference at low numbers of encounters (refer back to Figure 2.1). For higher numbers of predator encounters, $N_p \geq 0.5N_w$, predator memory is able to make a substantial difference. Increasing memory reduces the value of x^{**}, because at higher memories the success rate is closer to peak for lower and lower levels of x, so the performance of workers and predators diverge (and cause the fitness maximum) at a lower value of x. The magnitude of the maximum is also reduced by increased memory, because the performance difference between workers and predators is necessarily reduced as predator memory approaches the memory capacity of workers. Similarly, as the number of predator encounters increases as a fraction of the number of worker encounters, fitness maxima are found at lower colony lateralities x, and are of lower magnitude.

Generally, modelling workers as if they had to learn the preferences of their cooperators within their lifetimes produced similar results to the case where colony workers' cooperative preferences were encoded genetically, though shifted slightly towards greater laterality (in accordance with equivalently decreasing N_w and m_w from infinity). This similarity was observed for those conditions where the number of encounters or the memory capacity of workers exceeded that of their predators. For the remainder of the results we will use the model that assumes within-colony coordination is genetically optimised (equivalent to peak prediction for worker learning).

2.3.3 Coordination coefficient

Previous results assumed that predictability to predators was just as fitness-negative as lateralization-based coordination with fellow workers was fitness-positive; in other words, predator attacks and worker cooperative interactions had approximately the

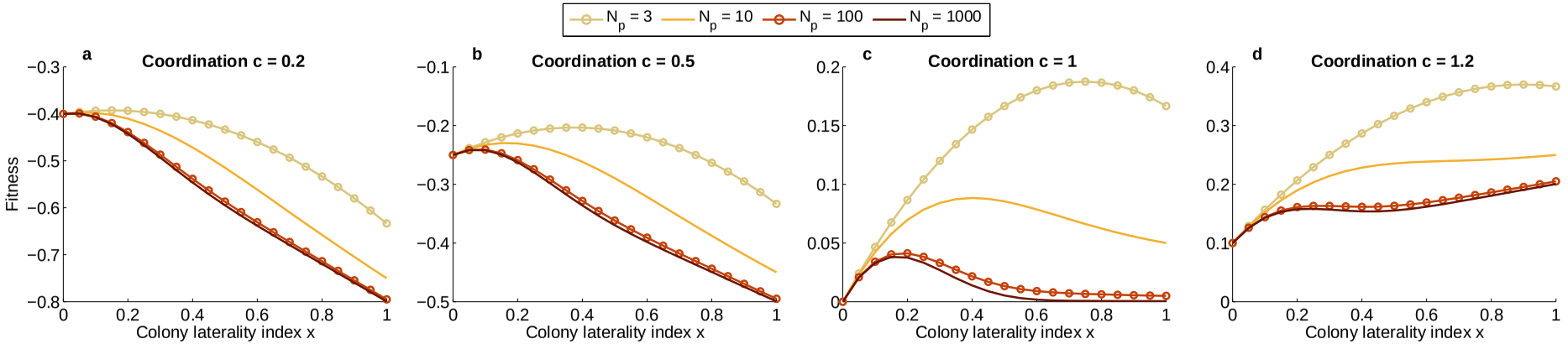

Figure 2.4: The impact of the importance of coordination c (a fitness multiplier applied to worker coordination success rates) on colony fitness. Results shown for predator memory length $m_p = 25$, and varying predator encounters N_p.

same evolutionary impact. Following Ghirlanda and Vallortigara (2004), we introduced a new term, c, the coordination coefficient, that determined the relative importance of worker-worker coordinated interactions and predator-worker interactions. We find that intermediate fitness maxima are produced when the coordination coefficient approximately satisfies $0.5 < c < 1.2$ (Figure 2.4). If coordination with fellow workers is sufficiently more important ($c \geq 1.2$), colonies are expected to be strongly laterally biased under most conditions because the advantage of being predictable dominates. On the other hand, if predator interactions become too important, $c \leq 0.2$, there is a strong incentive to have minimal lateral bias, in order to maximally confuse predators.

For any given pair of predator parameters N_p (number of encounters) and m_p (memory capacity), there is a broad range of colony lateralities that may be selected for by evolution (Figure 2.5). The optimal colony laterality x^{**} depends on the evolutionary importance of coordinated actions versus predator encounters, c. In each case, there is a threshold value of c below which colonies are expected to evolve towards fully random directional preference. There is also an upper threshold value of c, above which colonies are expected to evolve fully biased laterality, with all of their workers exhibiting the same preference. Between these two thresholds, colonies are expected to evolve mild to moderate degrees of laterality, $0 < x^{**} \lessapprox 0.5$ under most conditions. Optimal laterality x^{**} increases steadily with coordination coefficient c between the thresholds. Strong but incomplete laterality, $0.5 < x^{**} < 1$, may also be selected for here, but only when there are relatively few predator encounters ($N_p < 10$).

When predators prey on very few workers in their lifetime (e.g. $N_p = 3$) intermediate levels of lateralization can be selected across a wide range of c ($0.1 \lessapprox c \lessapprox 1.3$). As the number of encounters N_p increases, the lower threshold of c (below which evolution selects for fully random colonies) also increases. That is, as there are more encounters, a higher level of c is required to select for intermediate laterality bias. This is because increasing the number of encounters reduces the magnitude of the fitness peak (Figure 2.4). Higher c is therefore required to outweigh the benefit of random direction preferences as an anti-predator strategy.

When memory limits are more relevant ($N_p \geq 100$), increasing predator memory continues to increase the lower threshold of c. This happens for the same reason as increasing N_p: improving performance of the predator makes the magnitude of the fitness curve smaller because fitness is the difference between predator performance and peak performance (equivalent to genetically optimised directional coordination). The smaller fitness curve again needs a higher value of c to select for intermediate laterality. When the number of encounters and memory capacity of predators are both very high ($N_p = 1000$, $m_p = 1000$), intermediate lateralization is not selected

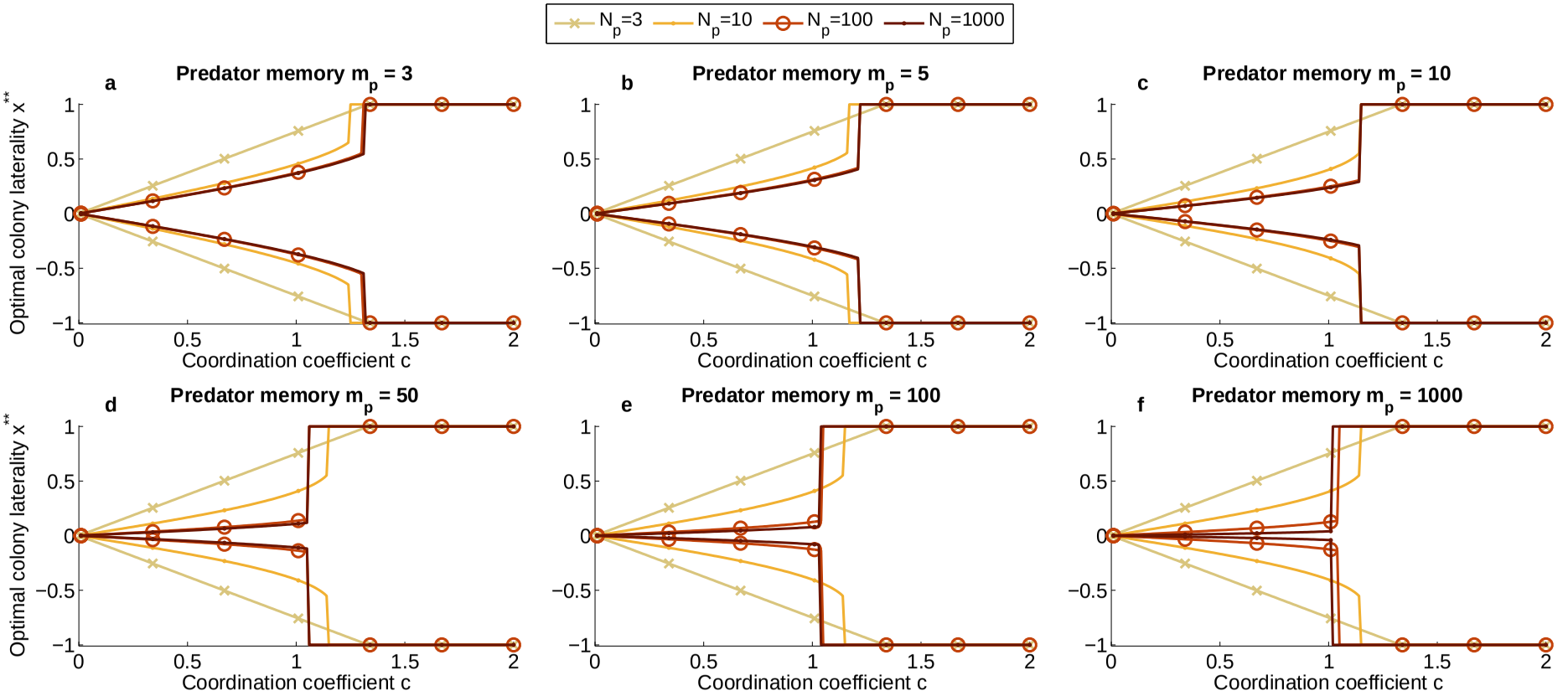

Figure 2.5: The impact of coordination coefficient c on optimal colony lateralization. Results shown for different numbers of predator encounters N_p and memory lengths m_p.

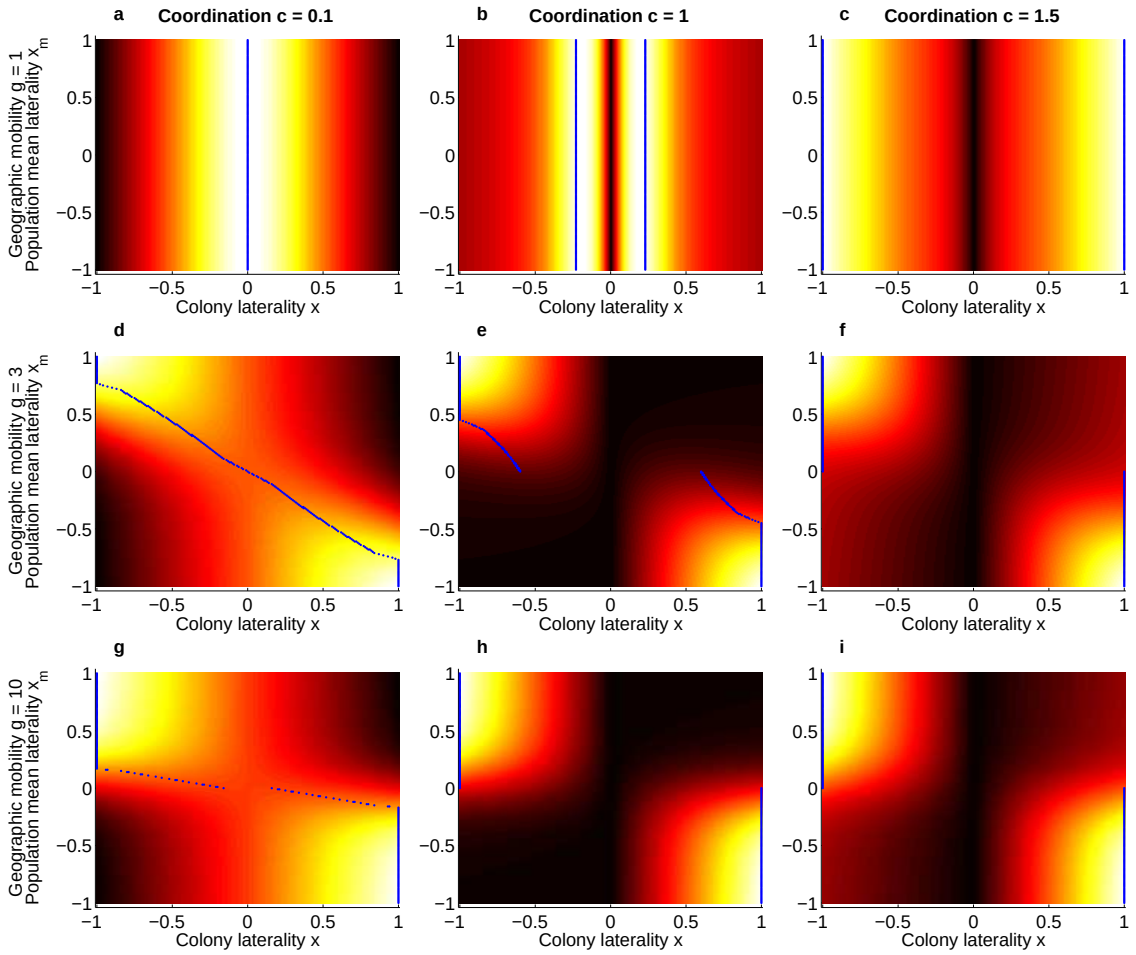

Figure 2.6: Colony fitness in a population where predators visit multiple colonies. Results shown for increasing numbers of colonies visited by the predator g (rows) and coordination coefficients c (columns). $N_p = 50$, $m_p = 25$. Blue dots indicate per-row global maxima.

for (Figure 2.5f): $c < 1$ produces zero lateralization, and $c > 1$ produces full lateralization. This is because, with such high values, predator performance is almost exactly equal to peak performance, so the fitness difference between workers and colonies at a given laterality is essentially determined by the value of c. Whether c is less than or greater than 1 therefore determines whether colonies should be fully random or fully laterally biased respectively.

Generally, the fitness advantage of intermediate lateralization is maximised when predator encounters and memory capacity are low, and this greater fitness advantage makes the result more robust to changes in the relative evolutionary importance of cooperation and predation, c.

2.3.4 Predation on multiple colonies

We next considered the case of local competition between colonies in a patch. For this it is important to consider not just the strength of a colony's laterality bias, but also its direction, because some colonies may be biased in the opposite direction to some others. The laterality index now ranges from $x = -1$, fully left-lateralized, via $x = 0$, fully random, to $x = 1$, fully right-lateralized. Transitioning to a multi-level model, where we consider a population of colonies, may introduce fitness interactions between colonies: the population mean lateralization x_m may affect the fitness of a given colony of laterality x. We focus on population-level predation effects by extending our model to allow predators to prey on multiple colonies at once. We measure the performance of colonies when their predators prey on some number of colonies with mean laterality x_m, as well as the given target (or *focal*) colony of laterality x. The total number of colonies that a predator visits on average is termed the geographic mobility of the predator, g.

When $g = 1$, each predator that preys on the focal colony with laterality x visits only that colony, so the population mean, x_m, does not affect colony fitness (Figure 2.6a-c). The results therefore mirror those of Figure 2.4, with peaks in the fitness surface found at identical degrees of lateralization, though for both positive and negative signs (see row-wise global maxima in blue, Figure 2.6a-c). When the coordination coefficient c is low, colonies with zero lateralization are favoured (blue dots, Figure 2.6a); when $c = 1$, intermediate lateralization is favoured (Figure 2.6b); when c is high, full lateralization is favoured (Figure 2.6c). However, when predators visit more than one colony, the global maxima change substantially.

The most robust effect is observed at the four corners of each fitness map (Figure 2.6d-i). When the population mean laterality x_m and the focal colony laterality x are strong, there is a substantial incentive for the focal colony to have the opposite laterality to the population mean. This is because predators' guesses will be influenced by, and thereby biased towards, the population mean. Consequently, to maximise fitness, colonies should contain individuals of the opposite lateralization, who are least vulnerable to predators that specialise on the majority lateralization. Concretely, if the predator specialises more on left-biased prey because they are substantially more common in the rest of the population, then having strongly right-biased workers will help evade attacks in a greater number of encounters. This produces a 2x2 chequerboard effect on each fitness map, though its intensity varies depending on the level of coordination and the number of colonies a predator typically preys upon.

When coordination is relatively unimportant ($c = 0.1$), increasing the geographic mobility of predators, such that they visit multiple colonies in a lifetime, introduces

a negative relationship between the mean population laterality x_m and the optimal colony laterality. For example, when predators visit 3 colonies on average ($g = 3$), there is an approximately linear relationship starting at $x_m \approx 0.75$, where the optimal colony laterality is at $x = -1$ (Figure 2.6d). The optimal laterality passes through the origin, $x^{**} = x_m = 0$, and ends at the point where the optimal colony laterality is at $x = 1$ for $x_m \approx -0.75$. Note that there are threshold population-level laterality strengths at either extreme: optimal colony laterality x^{**} is clamped at -1 where the population mean laterality $x_m \gtrapprox 0.75$, and $x^{**} = 1$ where $x_m \lessapprox -0.75$. That is, if the population is more than three-quarters left-biased, colonies consisting of exclusively right-biased individuals are favoured, and vice versa.

This is a weak manifestation of the aforementioned chequerboard effect. At greater coordination coefficients c and geographic mobilities g, the chequerboard effect is stronger, and clamping occurs at increasingly weaker population mean lateralities (compare e.g. Figure 2.6d with Figure 2.6e and g). Consequently, many more (and often most) conditions select for extreme colony lateralities of $x = \pm 1$ (vertical edges). Under these conditions, colonies that are less than fully biased against the population mean only begin to be selected for when the population mean is near to 0.

For intermediate values of c and g, there are a range of conditions – between the thresholds – where the optimal colony laterality is not ± 1 (Figure 2.6d,e,g). This occurs where the population laterality is somewhere between zero and the threshold. The strength of the optimal laterality, $|x^{**}|$, weakens as the population mean, x_m, moves further from its threshold value towards $x_m = 0$ (no population-level bias). This is because the advantage of being strongly lateralized in the opposite direction to the population majority is weaker when that majority is smaller. How much weaker the optimal laterality gets at $x_m = 0$ depends upon the mobility of the predator g and the importance of colony coordination c. For low g and c, optimal laterality $x^{**} = 0$ when there is no population-level bias (at $x_m = 0$, Figure 2.6a,d). As either g or c increases, evolution favours colonies with intermediate levels of lateralization. For example, $x^{**} \approx \pm 0.6$ when $g = 3$, $c = 1$ (Figure 2.6e), and $x^{**} \approx \pm 0.1$ for $g = 10$, $c = 0.1$ (Figure 2.6g). This is because when coordination is not important enough, maximally confusing predators is rewarded, anchoring optimal colony laterality at zero. Increasing the importance of coordination correspondingly increases the optimal laterality strength because more strongly lateralized colonies coordinate more effectively. Increasing the geographic mobility of predators can also increase the optimal laterality by reducing the importance of maximally confusing predators. This occurs because when predators interact with many colonies, and the population mean $x_m = 0$, predators will predominantly be interacting with individuals whose directional preferences do not exhibit any regularity on average –

weakening the ability of the predator to successfully prey on a given colony.

The chequerboard effect is essentially total when g and c are high enough. For example, when $g = 3$ and $c = 1.5$ (Figure 2.6f), the optimal colony laterality is found at the extremes ($x^{**} = \pm 1$) whenever $x_m \neq 0$. At high values of g, with predators preying on a greater number of colonies, predator learning is even more significantly influenced by the population mean x_m. This exaggerates the advantage conferred on colonies that are biased in the opposite direction, allowing even small population-level deviations from $x_m = 0$ to result in substantial colony-level incentives to oppose the population mean. High values of c, the coordination coefficient, contribute to the chequerboard effect by favouring colonies with stronger laterality biases – because strongly biased colonies are hypothesised to coordinate more effectively. This pushes the highs of the fitness surface out towards the left and right edges, exaggerating the peaks at the top left and bottom right of each fitness map.

From these fitness maps we can also infer whether any population-level bias would be expected. That is, in the metapopulation of colonies, should we expect more right-biased colonies than left-biased, vice versa, or an even distribution? If the population mean laterality is negative, $x_m < 0$, then the fittest colonies are found at positive colony lateralities, $x > 0$ (global maxima in blue, Figure 2.6d-i). Symmetrically, when $x_m > 0$, the fittest colonies are found at $x < 0$. Consequently, in the metapopulation of colonies, an over-representation of negatively-biased colonies will favour positively biased colonies and vice versa, stabilising around a even distribution of negative and positively biased colonies ($x_m = 0$), where colony fitness is equal for both sides. This is an instance of negative frequency-dependent selection creating a stable metapopulation-level equilibrium. Anti-predator adaptation is the source of this relationship; predators perform better against colonies that are towards the majority lateralization. Considering predators that visit more than one colony changes the expected absolute laterality $\mathbb{E}[|x|]$, but not the stability of the population mean $x_m = 0$, because negatively and positively biased colonies are affected symmetrically.

Because the metapopulation is expected to maintain an equilibrium with no population-level lateralization, $x_m = 0$, it may be fruitful to focus on the optimal colony-level lateralization at $x_m = 0$, and consider how this changes with other parameters of the model. These results correspond to predictions for the degree of colony-level lateralization expected in natural populations.

When there is no population-level bias, the geographic mobility of the predator, g, strengthens the optimal lateralization under almost all conditions (compare Figure 2.7a-b with c-h). This generally shifts the curves left, predicting intermediate levels of lateralization at smaller coordination coefficients. Once predators become more mobile, $g \geq 10$, many curves become horizontally contracted towards the

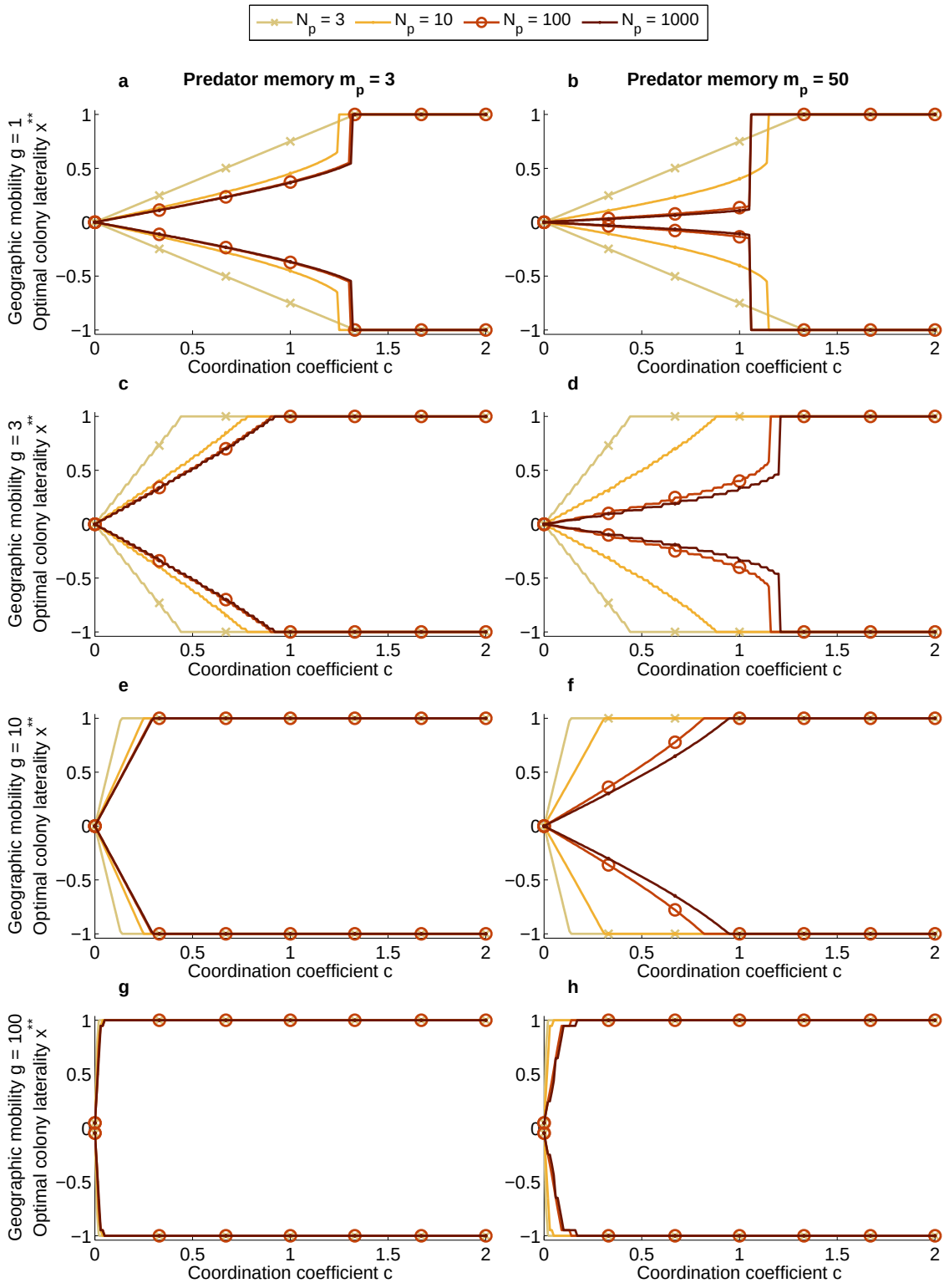

Figure 2.7: The impact of the geographic mobility of the predator g (rows) and the coordination coefficient c on optimal colony lateralization when there is no population-level lateralization, $x_m = 0$. Results shown for different numbers of predator encounters N_p (line series) and memory lengths m_p (columns). Note that the high frequency steps in the continuous lines (particularly visible in **c**, **d** and **h**) are an artefact owing to a computational simplification/optimisation.

left, because at $c = 0$, no colony-level bias is predicted, yet very small increases in the coordination coefficient are sufficient to ensure the evolutionary advantage of fully lateralized colonies (Figure 2.7e-f). When predators visit very many colonies, $g = 100$, a vast majority of conditions select for fully biased colonies, with lower lateralities only selected for when coordination has close to zero fitness implications, regardless of memory or numbers of encounters (Figure 2.7g-h).

The higher memory and encounter conditions are unique in experiencing a horizontal broadening, rather than narrowing, as geographic mobility increases modestly from the single colony case (compare Figure 2.7b and d, $N_p \geq 100$). Specifically, intermediate lateralization can be selected at higher coordination coefficients when predators visit three colonies on average rather than one. This is because predators will find it harder to learn and exploit mild lateralization in the focal colony when it is also interacting with individuals from another two colonies that have no lateralization. Once $g \geq 10$, predators' ability to exploit lateralization is determined predominantly by its interactions with the (unlateralized) population at large, so even strongly lateralized colonies do not suffer from predation costs very severely, because predators are sufficiently confused by the rest of the population. Evolution therefore selects for strong colony-level biases, which are also helped by the coordination advantage.

This boost to the fitness of mildly lateralized colonies does not occur under low memory or numbers of encounters, because predators are less able to pick up on and exploit small laterality biases, diminishing the fitness differences responsible for the effect. This also explains why the low memory and encounter conditions are more prone to the leftward contraction observed as g increases.

2.3.5 Bayesian predator

The results presented here were for simulations of a frequentist predator, which was not able to evolve a particular bias towards the left or right over multiple generations, but was instead restricted to within-lifetime learning and specialisation. Through additional simulations and mathematical reasoning, we determined that a Bayesian predator would perform identically to a frequentist predator with infinite memory – regardless of the shape of the Bayesian predator's prior – so long as the mean of the prior distribution was $\frac{1}{2}$ (data not shown). At the evolutionary equilibrium, where frequency-dependent selection drives population-level lateralization down to zero, the predator's optimal mean prior is indeed $\frac{1}{2}$, so a Bayesian approach would not affect the long-term evolutionary outcome. If a Bayesian predator were to co-evolve its prior with the evolution of the population of colonies, the number of generations to settle down to the equilibrium may change because predators would be able to

exploit biased populations through both lifetime learning and an evolved prior bias. However, owing to frequency-dependent selection, the equilibrium itself would not be altered.

2.4 Discussion

We investigated the evolutionary pressures on eusocial insect lateralization that operate above the individual level. We find that a trade-off between predictability to predators (or prey, or other competitors) and coordination with fellow colony-mates can favour colonies that have neither fully random nor fully uniform directional biases. In fact, most conditions select for a mild bias towards one direction or the other at the colony level, when competitors are assumed to interact with one colony at a time. When they interact with a small number of colonies (2-10), frequency-dependent effects arise in the population of colonies. Under these circumstances, we find that mild laterality biases may be selected for under a broader range of conditions if competitors' memory and numbers of encounters are moderate (at least 100 or so encounters over a lifetime, with competitors remembering their last 50 encounters). When competitors interact with many colonies at once (100 or more), e.g. a very geographically mobile predator, the detrimental effect of being strongly lateralized is all but eliminated. This means that even if coordinated actions have relatively little evolutionary significance (with a low coordination coefficient), colonies are predicted to be very strongly lateralized. Despite predicting colony-level bias under many conditions, our results do not predict a population-level bias; we would expect an equal proportion of right-biased and left-biased colonies.

2.4.1 Correspondence to *Formica rufa*

Returning to the example of the wood ant, *Formica rufa*, colonies in natural populations have been observed to have a laterality bias of magnitude $|x| = 0.2$ in a gap-crossing task that tests forelimb preference (see Appendix A). This degree of colony-level bias is predicted by our model under a number of conditions. It is possible when the evolutionary importance of lateralization-based coordinated actions is approximately equal to that of lateralization-based predation/opposition, or down to about half as important, depending on the competitor's memory and average number of encounters. Weak lateralities of 0.2 are most viable when competitors interact with a small number of colonies at a time (less than 10), which may be the case for the common wood ant "predator" class – parasitoids – due to their short lifespans (Weisser et al., 1994; Godfray, 1994). Ants have also been found to exhibit coordinated laterality in trophallaxis, route following and nest site explor-

ation (Frasnelli et al., 2012a; Heuts et al., 2003; Hunt et al., 2014) providing an existence proof for the class of lateralized actions on which our coordination effect is based.

2.4.2 Population-level bias

Our model predicts there should be no population-level lateralization because of negative frequency-dependent selection between colonies. If a majority of colonies were left-biased, for example, competitors may specialise on encountering left-biased workers, and the fitness of right-biased colonies would increase. Over a number of generations, right-biased colonies would be expected to increase in frequency, and if they subsequently came into the majority, left-biased colonies would then gain the advantage. The result is balancing selection, settling at a population-level equilibrium with equal proportions of right-biased and left-biased colonies.

While antagonistic interactions with other species are the drivers of this effect in our model, other forms of population-level competition between colonies are imaginable. In humans, Raymond et al. (1996) propose the "fighting hypothesis": that left-handers should have an advantage in fights and aggressive interactions over right-handers because right-handers are less familiar with facing left-handers (who are in a significant minority), yet left-handers are well-practised in facing right-handers. Supporting this hypothesis, Raymond et al. (1996) observe that left-handers are strongly over-represented in interactive sports (such as boxing and tennis), but not in non-interactive sports (such as swimming and bowling).

The logic of the fighting hypothesis may also apply to some eusocial insect species. For example, in *Formica rufa*, colonies compete with each other over territory in the early months of their founding (Skinner, 1980). These skirmishes have substantial ecological significance, because they help determine access to vital resources that will affect colony productivity for years to come. Indeed, informal observations indicate territory size in *F. rufa* is associated with the number of sexuals produced by the colony (Skinner, 1980). As in the fighting hypothesis, if one colony was strongly left-biased while the others that it encountered were right-biased, the left-biased colony may have greater success, being harder to predict by its foes. Similar to our model of predation and general inter-species antagonism, this effect would also create negative frequency-dependent selection, providing another possible mechanism for the maintenance of zero population-level bias. Though it should be noted that direct, interactive competition of this kind may not be widespread in eusocial insects.

2.4.3 Comparison to current theory

Prevailing theory predicts more social species to be more strongly lateralized at the population-level (Ghirlanda and Vallortigara, 2004; Ghirlanda et al., 2009). This is because individuals engaged in coordinated actions should benefit by having similar lateralization to those they are coordinating with. Conversely, we find that eusocial insects, some of the most social species on Earth, should have zero population-level lateralization, with evolution maintaining this zero bias through negative frequency-dependent selection on the direction of colony lateralization. This contradiction occurs because of the levels of selection. The prevailing theories are based on non-eusocial species, but eusocial insects have a completely different reproductive structure: unlike most group-living species, they may be deemed subject to group adaptation (Gardner and Grafen, 2009). Because the interests of most organisms are largely aligned within the colony we may expect evolution to optimise for colony-level success in many cases. It is in every worker's genetic interest to improve the efficiency of the colony, with within-colony antagonism gaining them little. For this reason, some find it helpful to consider eusocial colonies as superorganisms (Wheeler, 1911; Hölldobler and Wilson, 2009; Gardner and Grafen, 2009).

For population-level bias to be detected in a population of eusocial colonies, either the proportion of left- and right-biased colonies would have to deviate from 50:50, or colonies biased in one direction would need to be more strongly lateralized than those biased in the other direction (so the population mean laterality skews towards the stronger). No disparity in laterality strength between the two directions is expected, because left and right are presumed symmetric, offering no inherent advantage. And as previously discussed, we predict negative frequency-dependent selection to maintain an even proportion of left- and right-biased colonies. This negative frequency-dependent selection is seen in previous (non-eusocial) models too (Ghirlanda and Vallortigara, 2004; Ghirlanda et al., 2009), but is then offset by advantages conferred on those of majority-type lateralization when participating in coordinated or cooperative interactions (an instance of *positive* frequency-dependent selection). Returning to the example mentioned in the introduction, shoaling fish that coordinate to turn in the same direction as the majority may be less likely to be picked out by a predator (Ghirlanda and Vallortigara, 2004). This trade-off between minority advantage and majority advantage faced by individuals in non-eusocial populations creates a range of predicted lateralization, with those species engaged in more coordinated interactions expected to have stronger population-level lateralization. However, while coordinated interactions are very important *within* eusocial colonies, there is no evidence of cooperation or coordination *between* eusocial colonies. Eusocial colonies are actually in strong competition with one another.

Unopposed by the positive frequency-dependent selection found in models of non-eusocial species, we therefore expect negative frequency dependence to maintain even proportions of left- and right-biased colonies, producing zero population-level lateralization in species of eusocial insects.

2.4.4 Implications for empirical work

Existing theory (Ghirlanda and Vallortigara, 2004; Ghirlanda et al., 2009) has been very influential, driving recent empirical work on population-level lateralization (Anfora et al., 2010; Frasnelli et al., 2012a). In particular, the hypothesis that more social species, which undergo more coordinated or cooperative interactions, should have stronger population-level lateralization has been tested by looking at lateralization in closely related insect species who nevertheless differ in their degree of sociality (Anfora et al., 2010; Frasnelli, 2013). In particular, the population-level lateralization of eusocial species have been compared to that of nearby solitary species on the tree of life. However, as we have argued, the existing theory should break down for eusocial species. Intriguingly, empirical work seems to support the original hypothesis. One possible explanation is that the results that measured population-level lateralization were actually detecting colony-level lateralization. This empirical work typically samples from a single colony (Frasnelli et al., 2012a), or a small number of colonies (Letzkus et al., 2006; Anfora et al., 2010), so any population-level bias present in these samples may in fact be a by-product of colony-level bias, and if a larger number of colonies were sampled, population-level bias may disappear. Taken in this light, the existing empirical work could be seen as a test of both our work and that of Ghirlanda and Vallortigara (2004): in solitary species, we expect few coordinated interactions and therefore minimal laterality according to their work, and in eusocial species, we expect many coordinated actions *within* colonies, creating strong *colony*-level lateralization, according to our work.

All that said, our model is not completely incompatible with population-level bias. When predators visit only one colony on average, the frequency-dependent effect at the colony level disappears, so fitness is neutral between the two directions of colony-level bias, irrespective of the make-up of the population (Figure 2.6a-c). Genetic drift could then be expected to drive out one direction or the other, homogenising the direction of the population's bias, if the direction of lateralization were under genetic control (not just its strength, as in e.g. mice paw preference (Collins and Glick, 1985)).

Going forward, it is particularly important to determine whether existing measurements of population-level lateralization in eusocial insects are a by-product of colony-level lateralization. Without such a clarification, the observations to be ex-

plained remain ambiguous, and we cannot approach a causal explanation of lateralization in eusocial insects. A more comprehensive understanding of lateralization at multiple levels in all taxa could be attained through comparisons among species that share the same degree of sociality (be they eusocial or solitary), but yet differ in the expected number or importance of social interactions. Such experiments would get us closer to evaluating the coordination coefficient c and its effect on lateralization in asocial and eusocial species: the core hypothesis explored by Ghirlanda and Vallortigara (2004), and in this chapter.

2.4.5 Group selection on binary state characters

While our investigation has focused on lateralization, and predators' ability to learn and exploit the lateralization of colony workers, our results can also offer insight into the distribution of other binary traits at the individual, colony, and population levels in eusocial species. Correlated suits of traits exhibited by organisms, "animal personalities" (Wolf and Weissing, 2012), can often be summarised by binary traits (Riechert and Jones, 2008, for example). A well-studied example of animal personality is the boldness/shyness continuum (Sih et al., 2004). Whether prey are bold or shy could certainly impact the attack strategy of a predator, so if our model captures something general about predictability to predators, prey, or other competitors, it may extend to such cases. However, the coordination effect employed in our work would need some reconsideration, because personalities are often thought to be complementary to one another due to division of labour (e.g. *Anelosimus studiosus*, Holbrook et al., 2014; Chapter 3, this thesis). Consequently, fitness would not be maximised (*ceteris paribus*) by homogeneity, as we assume it is for lateralization. A thorough investigation into animal personality and predator/competitor prediction would need to consider the advantages of division of labour – and having traits distributed in certain proportions – versus the impact these proportions would have on predictability to predators and competitors.

2.4.6 Conclusion

By reconsidering the levels of selection on lateralization, we have overturned a prediction made by established theory (that more social species should be more strongly lateralized at the population level (Ghirlanda and Vallortigara, 2004; Frasnelli et al., 2012b)), offered a reinterpretation of existing empirical work, and identified avenues for future experiments. Failing to examine the effects of natural selection on a particular level in the biological hierarchy (here, colonies) can clearly have substantial consequences for the evolutionary analysis, and lead to the misinterpretation of experimental results. Fortunately in this case, previous empirical work is just as

relevant to the investigation at hand, but carries substantially different implications for our understanding once the levels of selection are properly accounted for.

A key assumption of this chapter was the minimal role of within-colony conflict, owing to eusociality and its genetic structure. In the next chapter we consider a colonial species that is more primitively social, the 'sub-social' comb-footed spider, *Anelosimus studiosus*. In this species of spider, individuals live in colonies, but all members of the colony reproduce: there is no reproductive division of labour, a defining characteristic of eusocial species. Without this, *A. studiosus* are predicted to be more prone to within-colony conflict than the eusocial species examined in this chapter. We therefore consider individual incentives more closely and assess whether they are aligned with, or in conflict with, the evolutionary interests of colonies. We model evolution explicitly at the individual level to determine whether individual selection could provide an alternative explanation for the distribution of aggressiveness among *A. studiosus* colonies, which was previously claimed to be the result of natural selection operating at the group/colony level (Pruitt and Goodnight, 2014). Thus, we approach the question of whether group selection can lead to group adaptation by investigating another well-studied biological system using theoretical methods.

Chapter 3

Group composition in sub-social spiders

This chapter is based on a paper under review with *Ecology & Evolution* (Calcraft et al., submitted[a]). An earlier version of this work was presented at *Mathematical Models of Ecology & Evolution*, Paris, France, 2015.

Abstract

Group selection is the selection of traits through the differential success of groups of organisms. Most evolutionary biologists agree that group selection can occur and is equivalent to kin selection for most purposes, but claims of group *adaptation*, where a trait evolves predominantly in the interests of the group, are often contested. A recent study of a sub-social spider species, *Anelosimus studiosus*, claimed to provide experimental evidence that group selection had resulted in group adaptation. *Anelosimus studiosus* lives in multi-female colonies, some of whom are aggressive and some of whom are docile. It is the colony's docile:aggressive ratio, and its relationship with colony size and resource availability, that is claimed to have evolved via group selection. By extending the classical Hawk-Dove model from evolutionary game theory, which closely matches the species' aggressive-docile dimorphism, we predict levels of aggression based on individual incentives alone. Considering the impact of colony size and prey availability on competition for resources, we show that frequency-dependent individual selection can also explain the experimental observations. Consequently, both individual and group selection are likely to be operating in the same direction. However, because the original study did not measure within-group fitness, the relative strengths of these two forces remains unknown. Therefore, group adaptation has not yet been demonstrated in this sub-social spider.

3.1 Introduction

Group selection occurs when groups of organisms experience differential success owing to a heritable trait of the group (e.g. proportion of altruists), amounting to Darwinian competition between groups (Wilson and Wilson, 2007). In some circumstances, group selection may lead to group adaptation – the evolution of traits in accordance with the interests of groups – but this remains controversial (Gardner and Grafen, 2009; Sober and Wilson, 2011; Gardner, 2015; Pruitt and Goodnight, 2015).

A recent study (Pruitt and Goodnight, 2014) of a sub-social spider species, *Anelosimus studiosus*, claimed to provide experimental evidence that group selection drove the evolution of aggressiveness in spider colonies, resulting in group adaptation. The study has received a number of criticisms: methodological (Grinsted et al., 2015), theoretical (Gardner, 2015; Smallegange and Egas, 2015; Krupp, 2016; Biernaskie and Foster, 2016) and semantic (Gardner, 2015). While many of these critiques stress that individual-level selection, rather than group selection, could be responsible for the observations (Grinsted et al., 2015; Smallegange and Egas, 2015; Krupp, 2016; Biernaskie and Foster, 2016), no satisfying biological explanation for the phenomena has been offered to support this view. A number of alternative models have been proposed, but they are either highly abstract, incorporating little of what is known about the biology and ecology of *A. studiosus* (Smallegange and Egas, 2015; Gardner, 2015), or they neglect the role of within-group selection against aggressiveness (Gardner, 2015; Biernaskie and Foster, 2016), which is supported by previous evidence (Pruitt and Riechert, 2009).

In this chapter, we build an individual-level model of the evolution of aggressiveness in *A. studiosus*, integrating evolutionary game theory (Maynard Smith and Price, 1973; Maynard Smith, 1982) with previous research on this species' aggressive-docile polymorphism (Pruitt and Riechert, 2009) and empirical data regarding prey capture in a closely related colonial spider, *A. eximius* (Yip et al., 2008). *Anelosimus studiosus* is an interesting study system for the group selection controversy because, while these spiders live in groups and may be subject to group-level effects on fitness, they all reproduce as individuals. Without the reproductive division of labour found in eusocial species (Chapter 2), individual-level selection is expected to be strong, and is likely to be responsible for most organismic traits. Our investigation will therefore consider when individual selection can account for group-level phenomena, as well as the question of when group selection leads to group adaptation.

3.1.1 Group selection in *Anelosimus studiosus*

At higher latitudes, *A. studiosus* lives in mixed colonies of aggressive and docile females (Pruitt and Goodnight, 2014; Furey, 1998). The docile:aggressive ratio appears to depend upon the number of spiders within a colony (size) and the availability of prey (Pruitt and Goodnight, 2014). The increase in the number of aggressive spiders within colonies as they become larger is greater at high resource sites with higher prey availability than at low resource sites. Therefore, large colonies are highly aggressive at high resource sites, but are largely docile at low resource sites. Pruitt and Goodnight (2014) deployed colonies with artificially altered docile:aggressive ratios in high and low resource sites, and observed whether the docile:aggressive ratio affected colony survival. After two generations, they found that colonies were more likely to go extinct when their size and aggressive:docile ratio were furthest from those of naturally occurring colonies at the site at which they were deployed. This suggests there may be an optimal level of aggression for a given colony size, and that this level of aggression varies between high and low resource sites.

Such differential extinction of colonies (if methodologically sound, see Grinsted et al., 2015; Pruitt and Goodnight, 2015) constitutes group selection, but whether group selection played a key role in evolutionary *adaptation* is contested. Gardner and Grafen (2009) argue that the word *adaptation* should only be used when referring to an optimising process. Group adaptation should therefore only be said to occur when a trait is optimised solely for the group, and not compromised by the selfish individual interests of group members. Pruitt and Goodnight (2015) take issue with this definition of adaptation and suggest that 'group-level adaptation' occurs whenever group selection merely contributes to the evolution of a trait, even if the trait is also subject to individual selection. Sober and Wilson (2011), on the other hand, argue group-level adaptation should refer to cases where group selection is the more important force (compared with individual selection) in the evolution of a trait. We favour Sober and Wilson's (2011) definition, because it allows us to ascribe an adaptation to the individual or the group according to which entity's interests the adaptation predominantly serves.

3.1.2 Evolutionary game theory

Evolutionary game theory is the application of game theory (Von Neumann and Morgenstern, 1944) to evolutionary biology (Maynard Smith and Price, 1973; Maynard Smith, 1982). Game theory is used to model competitive interactions and predict the behaviour of rational agents whose interests typically conflict (Myerson, 1991). A central tenet of game theory states that we should consider whether "the best

strategy to adopt depends on what others are doing" (Maynard Smith, 1982). A game theorist first assumes each player employs one strategy from a specified set of alternatives (or a mix of strategies, using each with a certain probability), and then calculates the expected payoff to individuals using each strategy against each other possible strategy, according to the rules of the game (specified as assumptions of the model).

Game theory makes complex situations mathematically tractable. It can identify the best strategy to play under various conditions, and how any number of interacting rational agents, all attempting to maximise their payoffs, would act. Evolutionary game theory applies this to populations of organisms, predicting how different strategies would evolve by considering which would receive the highest evolutionary payoffs (fitness). Evolutionary game theory particularly pays attention to how different distributions of strategies within a population can affect the average payoffs of those strategies, and how those averaged payoffs can shape the future distribution of those strategies in the population through natural selection, subsequently affecting the future average payoffs, and so on. Theorists often look for an *evolutionarily stable state* (ESS), which is a distribution of strategies (a state of the population) that is robust to minor disturbances in the distribution of strategies (Maynard Smith, 1982). Because evolution will tend to maintain such a population state once it is reached, naturally occurring populations are expected to often be observed near to such an evolutionarily stable state.

Evolutionary game theory was first employed by Maynard Smith and Price (1973) to understand behaviour that appeared initially confusing from an individual perspective, particularly cases that had attracted group-level hypotheses to fill the explanatory void (Huxley, 1966; Wynne-Edwards, 1962). This is because game theory's focus on the performance of strategies relative to the distribution of other strategies allowed it to reveal counter-intuitive results of self-interest, such as individual restraint in intraspecies conflict (Maynard Smith and Price, 1973). Further, when the fitness of a behaviour depends on the evolved behaviour of others, frequency-dependent selection can maintain multiple strategies within a single population (Strickberger, 2000). Such a mix of individual strategies may appear to be top-down, group-level organisation such as task specialisation (Pruitt and Riechert, 2010; Wright et al., 2014). Here, we consider aggressive and docile as individual strategies within colonies of *A. studiosus* and develop a game-theoretic model to investigate colony composition using selection at the individual level.

3.1.3 The Hawk-Dove model

Under certain conditions, the Hawk-Dove evolutionary game (Maynard Smith and Price, 1973) predicts a stable equilibrium (an evolutionary stable state) with the coexistence of two phenotypes: an aggressive Hawk strategy willing to engage in costly fights to obtain contested resources, and a submissive Dove strategy that seeks to avoid agonistic encounters, sharing resources evenly with other Doves. This equilibrium is the result of frequency-dependent selection: a high frequency of Hawks favours Doves because they avoid excessive costly fighting, but a high frequency of Doves favours Hawks because they obtain more resources through aggression. This game matches observations of *A. studiosus*: aggressive spiders monopolise feeding in staged bouts with docile spiders (Pruitt et al., 2008) and dominate in 5-way encounters (Pruitt and Riechert, 2009), but lose their advantage at high frequencies through agonistic engagement (Pruitt and Riechert, 2009).

To explain the observed patterns of aggressive and docile spiders in *A. studiosus* colonies, we consider here how individual payoffs may change with colony size and prey availability, extending the classic Hawk-Dove evolutionary game with variable resource values to predict outcomes for large and small colonies at high and low resource sites.

3.2 Materials and methods

We built a Hawk-Dove model of individual fitness for *A. studiosus* colonies of variable size and resource level. The classic Hawk-Dove game (Maynard Smith and Price, 1973) uses a fixed resource value, V, played for in each encounter. We modified the game so that colony size, n, affected the average resource value fought over, $v\,(n)$, producing a revised payoff matrix (Table 3.1), where C is the cost of fighting for Hawks and must exceed $v\,(n)$.

	meets Hawk	**meets Dove**
Hawk	$\frac{v(n)-C}{2}$	$v(n)$
Dove	0	$\frac{v(n)}{2}$

Table 3.1: The payoff matrix for the extended Hawk-Dove evolutionary game. Encounters are played for the resource value, $v(n)$, which varies with colony size, n, and the level of resources in the environment. Hawks engage in fights when encountering other hawks, at a cost, C.

We assume $v\,(n)$ is equal to the average prey mass captured per spider per hour in the web. We model the effect of relative changes in available resource quantity across

different conditions, rather than examining absolute values. Within our framework, $v(n)$ represents the average resource value fought over in idealised one-on-one encounters, which explains the importance of considering prey mass per spider; the resource value available in these encounters will drop as the colony size grows, unless additional spiders in the colony contribute with additional prey capture.

Colonial spider species, such as *A. studiosus*, enjoy greater capture efficiency in larger colonies for a number of reasons. By cooperating, spiders can subdue larger prey items than they would be capable of alone (Yip et al., 2008). Additionally, prey may find it harder to escape the web when more spiders are present, as they "ricochet" from one spider to another (Uetz, 1989). Measurements of *A. eximius*, a close relative of *A. studiosus*, confirm that larger colonies capture increasing levels of prey biomass per capita (Yip et al., 2008). As no equivalent measurements have been made for *A. studiosus*, we model the resource value available in colonies of different sizes using this prey capture data from *A. eximius*.

The average biomass captured per spider per hour in colonies of size n can be split into the product of two quantities: the average number of prey items captured per spider per hour, $p(n)$, and the average mass of captured prey items, $m(n)$. *A. eximius* capture fewer prey items per capita as colony size increases due to the decreasing surface area-to-volume ratio of the web (Yip et al., 2008):

$$p(n) = F \cdot n^{-b_p} \cdot 10^{c_p} \tag{3.1}$$

where b_p and c_p are coefficients from Yip et al. (2008, see Table 3.2, this thesis). F is a linear scaling factor that may be used to adjust the number of prey that colonies can capture (Figure 3.1a).

Relationship	**Coefficient**	**Value**
Mean prey mass best-fit	a_m	-0.30
	b_m	2.20
	c_m	-2.37
Prey number per spider per hour best-fit	b_p	0.45
	c_p	-1.80

Table 3.2: Best-fit parameter values for prey mass and prey number relationships with colony size in *A. eximius* (Yip et al., 2008).

Larger colonies, however, capture larger prey items. As a consequence, the mean prey weight increases faster than the number captured decreases, producing a positive relationship between colony size and per capita biomass for colony sizes

< 700 (Yip et al., 2008). The relationship between mean prey mass and colony size, $m(n)$, for *A. eximius* is (Yip et al., 2008):

$$m_{\text{eximius}}(n) = n^{a_m \log_{10}(n) + b_m} \cdot 10^{c_m} \tag{3.2}$$

where a_m, b_m and c_m are coefficients from Yip et al. (2008) (Table 3.2). We consider the colony size range of between 1 and 140 spiders studied by Pruitt and Goodnight (2014). Extrapolating the best-fit curve from Yip et al. (2008), the average mass of a prey item captured by a single spider ($n = 1$) drops to unrealistically low masses below 0.01 dried mg. Therefore, we adjusted the curve to ensure it begins at a definite starting mass, m_1, for $n = 1$. Because the *A. eximius* prey item mass relationship is very nearly linear for colony sizes in the range 1-140, we replace it with a straight line relationship between the definite starting mass we impose, and the mass observed by Yip et al. (2008) for the maximum colony size of 140. Our adjustment therefore provides a minimal correction for the best-fit's near-zero artefact. Thus, the mean prey mass, $m(n)$, in our model is:

$$m(n) = m_1 + (n-1)k \quad \text{where} \quad k = \frac{m_{\text{eximius}}(140) - m_1}{140 - 1} \tag{3.3}$$

This relationship is illustrated in Figure 3.1b. The total prey mass captured per spider per hour is then:

$$v(n) = p(n) \cdot m(n) = F \cdot n^{-b_p} \cdot 10^{c_p} \cdot (m_1 + (n-1)k) \tag{3.4}$$

The equilibrium proportion of Hawks for a given colony size, $h^*(n)$, can be calculated from the payoffs in the Hawk-Dove game (Table 3.1) as $\frac{v(n)}{C}$. The fighting cost, C, affects the range of possible equilibrium Hawk proportions that our model can produce, but we cannot know its value because absolute fitness cannot be directly calculated. Therefore, we factor out C (which does not vary between conditions, *ex hypothesi*), and normalise $v(n)$ to the range $[0, 1]$, thereby ensuring our model can predict the full range of aggression, from fully docile to fully aggressive colonies:

$$h^*(n) = \frac{v(n) - \min[v(n)]}{\max[v(n)] - \min[v(n)]} \tag{3.5}$$

3.2.1 Basic model of prey availability

At high resource sites, we assume that prey number and prey mass relationships match those of *A. eximius*, and set the scaling factor F in Equation 3.4 to unity ($F = 1$). Thus, colonies catch prey of increasing average mass as colony size increases, capturing increasing levels of prey biomass per spider. We assume there will be fewer prey to capture at low resource sites and adjust F (< 1) accordingly, where F determines the fraction of prey mass available at low resource sites compared to high resource sites, and affects all colony sizes equally (Figure 3.1c). By varying F, we considered the implications of more or less extreme reductions in prey availability on the expected equilibrium level of Hawks, $h^*(n)$, for colonies of different sizes.

3.2.2 Expanded model of prey availability

The basic model captures uniform effects on prey availability, independent of colony size. In some cases, however, limited prey availability may affect large and small colonies differently. To model this, we introduced two alternative scaling factors, the first of which, F_p, affects the number of prey items captured per spider per hour, $p(n)$:

$$p(n) = n^{-F_p \cdot b_p} \cdot 10^{c_p} \tag{3.6}$$

When increasing F_p (> 1), the quantity of prey items captured per spider decreases more rapidly with increasing colony size, reducing the average biomass per capita for larger colonies (Figure 3.2a). When $F_p = 1$, $p(n)$ matches the Yip et al. (2008) data.

The second of the new scaling factors, F_m, affects the average mass of prey items captured, $m(n)$:

$$m(n) = m_1 + F_m \cdot (n-1)\, k \tag{3.7}$$

When decreasing $F_m (< 1)$, the average mass of captured prey items increases less rapidly with increasing colony size, reducing the cooperative advantage of larger colonies (Figure 3.2b). Again, when $F_m = 1$, $m(n)$ matches the Yip et al. (2008) data.

Together, the calculation of resource value, $v(n)$, in the expanded model is:

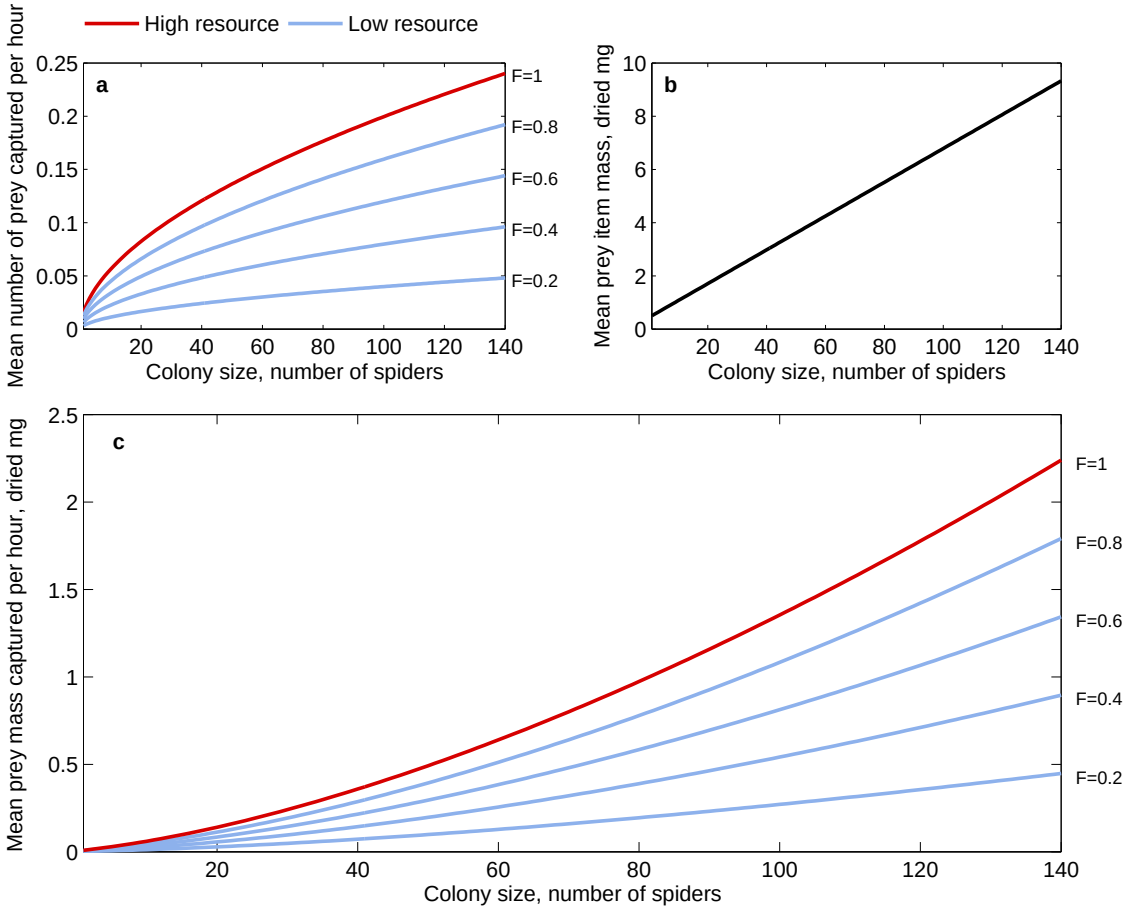

Figure 3.1: The effect of parameter F on prey capture in the basic model. (**a**) Average number of prey captured per hour (by the whole colony, $n \cdot p(n)$, Equation 3.1) for colonies of different sizes in high resource sites (red, $F = 1$) and a range of low resource sites (light blue, $F < 1$) in the basic model. (**b**) Average prey item mass ($m(n)$, Equation 3.3) for colonies of different sizes (assumed equal in low and high resource sites for the basic model). (**c**) Colony mean prey mass captured per hour ($n \cdot v(n)$, Equation 3.4) for colonies of different sizes in high resource sites (red, $F = 1$) and low resource sites (light blue, $F < 1$). Average mass captured by a singleton spider colony $m_1 = 0.5$ dried mg.

$$v(n) = p(n) \cdot m(n) = n^{-F_p \cdot b_p} \cdot 10^{c_p} \cdot (m_1 + F_m \cdot (n-1)\, k) \tag{3.8}$$

As in the basic model, the scaling factors were set to unity ($F_p = 1,\ \ F_m = 1$) at high resource sites. These factors were then adjusted at low resource sites to consider different combinations of mild and severe effects on both prey mass and prey quantity (Figure 3.2c). The model was then used to predict the expected level of aggression under Hawk-Dove equilibrium, $h^*(n)$, in colonies of varying size at both high and low resource sites.

F	Fraction of prey captured (number of prey per hour) in low resource sites compared to high resource sites, $0 < F < 1$ (basic model)
F_p	Modifier for prey capture quantity in low resource sites relative to high resource sites, $F_p > 1$ (expanded model)
F_m	Fraction of average prey item mass in low resources sites compared to high resource sites, $0 < F_m < 1$ (expanded model)
m_1	Mass captured by a singleton colony, dried mg

Table 3.3: Variables used in the basic and expanded models.

3.3 Results

In the basic model, F is the fraction of prey captured at low resource sites compared with high resource sites. The relative prey abundance at high and low resource sites was not comprehensively documented by Pruitt and Goodnight (2014), but the average number of struggling prey in colony webs over multiple observations, 0.9 at low resource sites and 1.8 at high resource sites, suggests a value of $F = 0.5$. When this difference is incorporated into the basic model, larger colonies at high resource sites are predicted to have higher numbers of aggressive spiders than those at low resource sites, matching the observations of naturally occurring colonies (Figure 3.3). This core prediction, of a steeper increase in aggressive spiders with colony size at high resource sites than at low resource sites, holds for a range of parameter values that represent moderate, but not extreme, reductions in prey availability at low resource sites (Figure 3.4a). When prey availability at low resource sites is between 0.3-0.6 of that at high resource sites, the model fits the original data well and the error is consequently lowest (Figure 3.4a-c). Note that the value of $F = 0.5$ suggested empirically (Pruitt and Goodnight, 2014) falls within this range. Prediction error

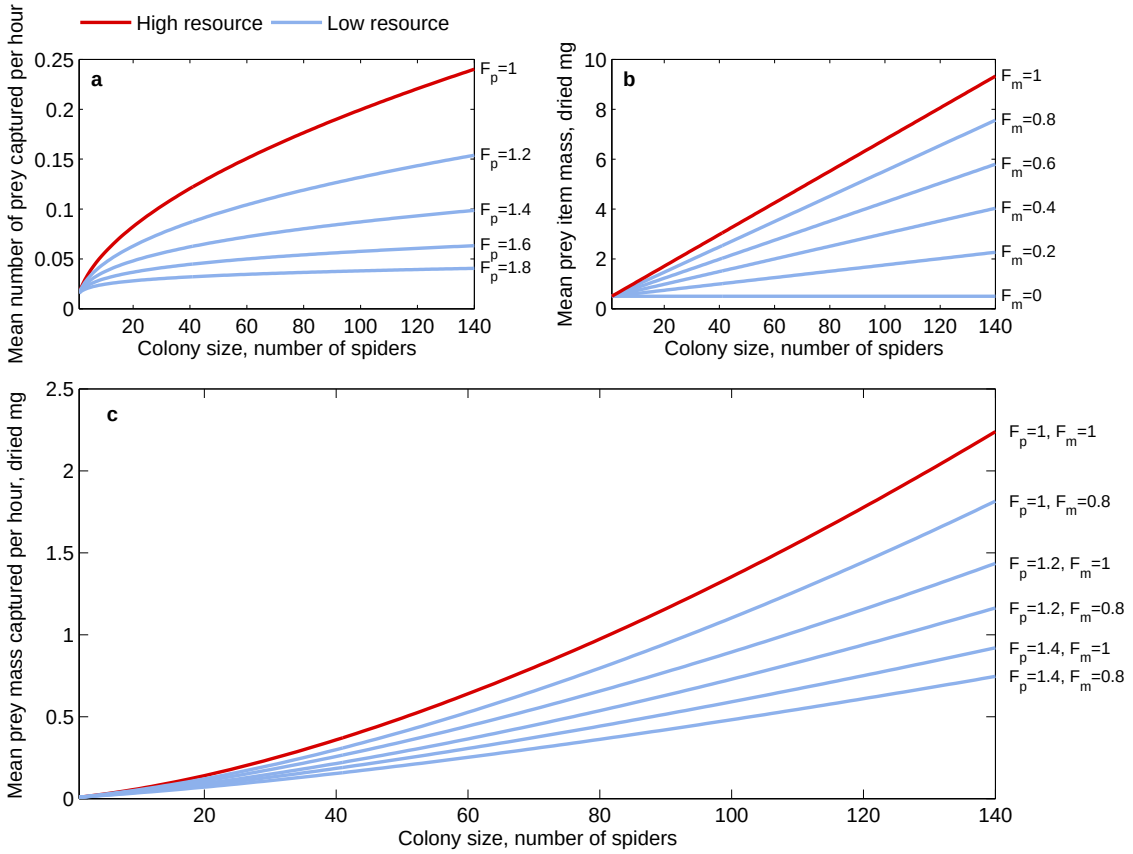

Figure 3.2: The effect of parameters F_p and F_m on prey capture in the expanded model. (**a**) Average number of prey captured per hour (by the whole colony, $n \cdot p(n)$, Equation 3.6) for colonies of different sizes in high resource sites (red, $F_p = 1$) and a range of low resource sites (light blue, $F_p > 1$) in the expanded model. (**b**) Average prey item mass ($m(n)$, Equation 3.7) for colonies of different sizes in high resource sites (red, $F_m = 1$) and low resources sites (light blue, $F_m < 1$). (**c**) Colony mean prey mass captured per hour ($n \cdot v(n)$, Equation 3.8) for colonies of different sizes in high resource sites (red, $F_p = 1, F_m = 1$) and low resource sites (light blue, $F_p > 1$ or $F_m < 1$). Average mass captured by a singleton spider colony $m_1 = 0.5$ dried mg.

increases when the average mass of prey items captured by a singleton spider colony, m_1, is greater than 1.3 dried mg. When individuals are able to capture such large prey, the resource value available per spider at high resource sites is greatest for very small colonies, rather than per capita resources increasing with colony size. Thus, predicted levels of aggression do not increase consistently with colony size at high resource sites, no longer matching the data (Figure 3.4d). However, prey items of such large mass may be unrealistic for individuals of *A. studiosus* to capture alone, being just 8mm in size themselves.

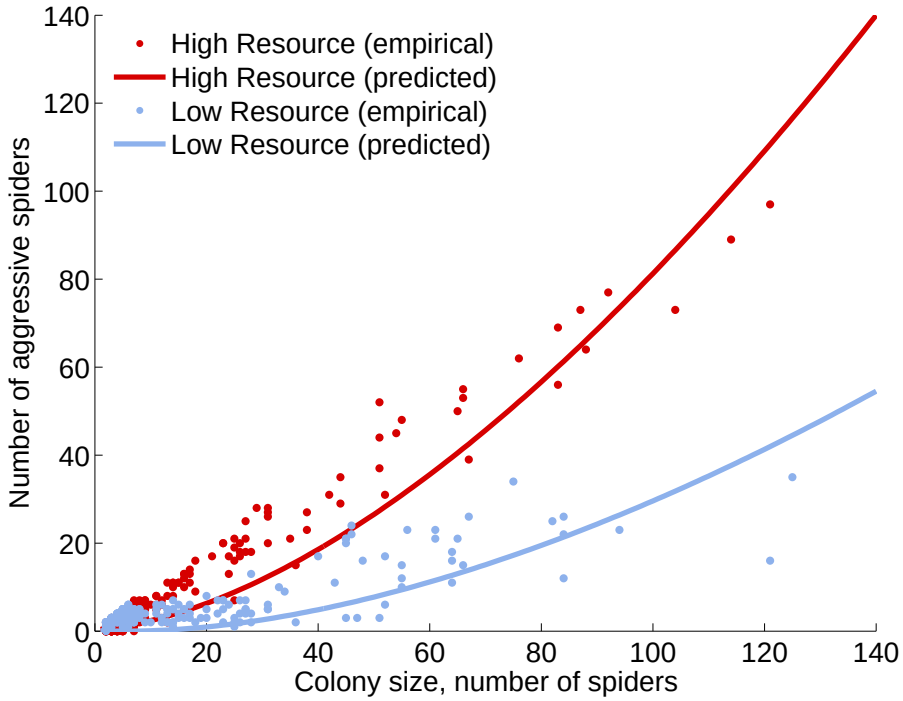

Figure 3.3: Resource availability can explain composition preferences through individual selection. In high resource sites, larger colonies are able to capture increasingly larger prey, supporting a greater number of aggressive spiders in the colony (red line). Limited prey availability in low resource sites leads to a shallower increase in biomass available, and therefore fewer aggressive spiders predicted as colony size increases than in high resource sites (light blue line). These predictions match the observed level of aggression in naturally occurring colonies at high and low resource sites (red and blue points, respectively), yet have not been explicitly fitted. Predictions shown here for the basic model, $F = 0.5$, $m_1 = 0.5$ dried mg. Experimental data replotted from Pruitt and Goodnight (2014).

The expanded model allows us to consider alternative effects of limited prey availability on colony capture through parameters F_p (affecting prey capture quantity) and F_m (affecting average prey item mass). At low resource sites, increasing $F_p > 1$ causes the number of prey captured per spider per hour to decrease more

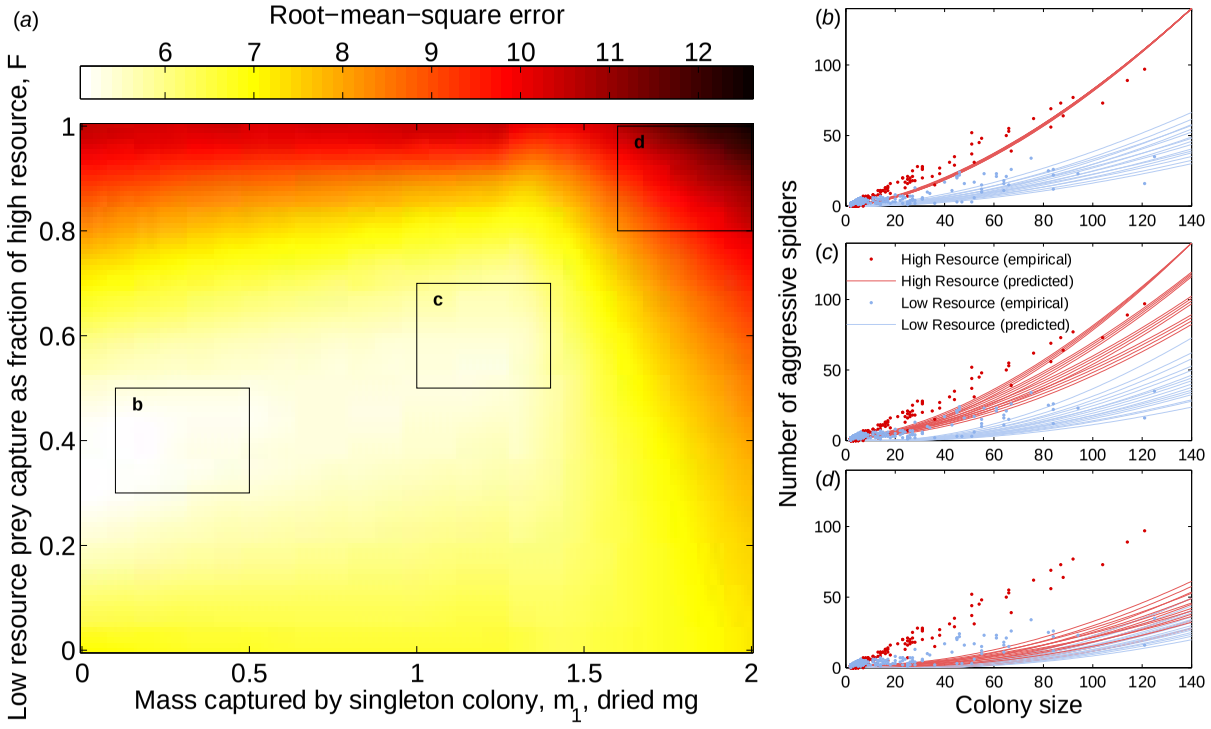

Figure 3.4: Goodness of fit between the basic model and empirical observations of naturally occurring colonies at high and low resource sites. (**a**) Root-mean-square error between the predicted number of aggressive spiders in colonies and the empirical data, shown over different values for model parameters: F, the resource availability in low resource compared with high resource sites (Equation 3.4), and m_1, the average prey item mass assumed to be captured by a singleton spider colony. Rectangles b, c and d show parameter regions whose corresponding fits to the data are shown in (**b-d**). (**b-d**) The number of aggressive spiders in colonies of varying sizes at both high and low resource sites (points), and our model predictions (lines) for an equispaced sample of parameter values from their respective regions in (**a**). (**b**) Model fits from a region of low error. (**c**) Wider variation in high resource site predictions from a region of intermediate error. (**d**) Model fits from the region of highest error. Experimental data replotted from Pruitt and Goodnight (2014).

rapidly with colony size, thereby attenuating aggregate colony capture per hour at larger colony sizes (Figure 3.2a). This scaling factor accounts for a variety of possible problems faced by large colonies at low resource sites. For example, the quantity captured by larger colonies may be affected more by their reduced surface area per spider when prey capture opportunities are sparse. Lowering the second scaling factor $F_m < 1$ reduces the rate of increase of average prey item mass with colony size (Figure 3.2b), which for instance models the case where low resource sites have a deficit particularly of larger prey items.

The expanded model makes similar predictions to the basic model when the scaling factors moderately reduce prey availability in low resource sites relative to high resource sites (e.g. $F_p = 1.2$, $F_m = 0.6$, Equation 3.8). The model thus fits the experimental data, and correspondingly has its lowest error in comparison to the empirical data, at such intermediate values (Figure 3.5). The model has higher error both when there is little difference between high and low resource sites ($F_p \cong 1$, $F_m \cong 1$), and when differences become too great ($F_p > 1.5$, $F_m < 0.5$).

Our models are robust to $\pm 50\%$ deviations in the coefficients a_m, c_m, b_p, and c_p, and to positive deviations in b_m of up to 50%. However, negative deviations of more than 10% in b_m disproportionately affect the prey mass relationship limiting the largest studied colonies ($n = 140$) to capturing prey items of less than a third of the mass predicted for the same size colony in *A. eximius* (Yip et al., 2008), thereby changing the predictions of our model substantially.

Both the basic and expanded versions of our model, built upon empirical data from *A. studiosus* and the closely related *A. eximius*, can therefore recapitulate the observed levels of colony aggression under a range of parameters, in the absence of group selection.

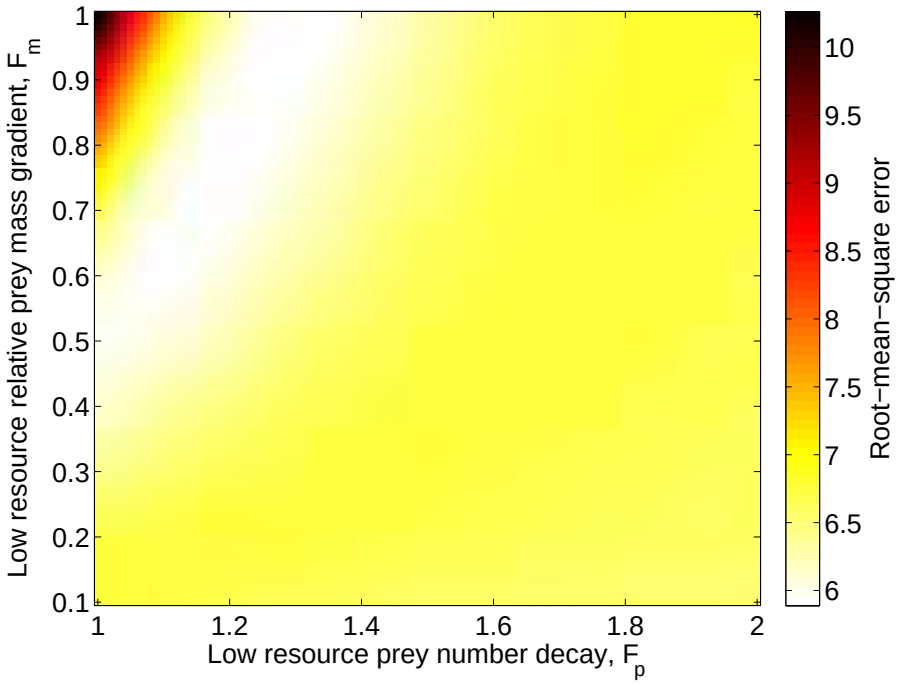

Figure 3.5: Root-mean-square error for predicted number of aggressive spiders in colonies at low and high resource sites in the expanded model compared with empirical observations of naturally occurring colonies. Error values are shown over different values of parameters that determine the severity of resource scarcity in low resource sites, $F_p \geq 1$ and $F_m \leq 1$. Where both parameters are 1, low resource sites and high resource sites are equal, and prediction error is greatest. Predictions assume the mass captured by a singleton colony, m_1, is 0.5 dried mg.

3.4 Discussion

We investigated whether patterns of colony aggression in *A. studiosus* could be explained by individual selection as an alternative to, or possible complement to, the group selection explanation offered by Pruitt and Goodnight (2014). We showed that a modified Hawk-Dove game, which assumes only that individual spiders are maximising their foraging success, can reproduce the relationships between size, resource level and aggression found in naturally occurring colonies (Pruitt and Goodnight, 2014). Our models fit the data for parameters that reflect a moderate difference between high and low resource sites, and are thus both predictive and falsifiable, while agreeing with the available evidence on prey abundance at the studied sites (Pruitt and Goodnight, 2014). We have, therefore, offered a biological explanation for the observed phenomena, something missing from previous individual-level models of this system (Smallegange and Egas, 2015; Gardner, 2015). High resource sites favour colonies that are increasingly aggressive as they grow because aggressive

spiders can capitalise on additional resources obtained through cooperative capture. Conversely, limited prey availability at low resource sites leaves larger colonies unable to provide for many aggressive spiders, favouring lower aggression than their high resource counterparts as colonies grow.

3.4.1 Group selection *versus* individual selection

Following the theoretical implausibility of naïve, early models of group selection (pre-1970s), Williams (1966) proposed his principle of parsimony that suggested individual selection explanations of biological phenomena should be preferable *a priori* to group selection explanations. However, the acknowledgement of the equivalence of group and kin selection (Price, 1970; Hamilton, 1975; Grafen, 1984; Marshall, 2011a), as well as general advances in the application of multi-level selection theory (Wilson and Wilson, 2007), have questioned this principle. Group and individual selection should be evaluated on a case-by-case basis, and the comparison of these two forces is fundamental to the study of multi-level selection (Wilson and Wilson, 2007; Eldakar and Wilson, 2011; Okasha, 2006). Measuring the relative strength of group and individual selection is even more important when asserting group *adaptation* (Sober and Wilson, 2011; Gardner and Grafen, 2009). Indeed, Pruitt and Goodnight (2014) claimed to demonstrate group adaptation in *A. studiosus* but were criticised for the absence of individual fitness measurements in their study (Grinsted et al., 2015; Smallegange and Egas, 2015; Krupp, 2016).

Previous empirical work has examined individual selection for aggressiveness in *A. studiosus*, finding a relationship between the number of aggressive spiders in a group and the fitness of the individuals in that group (Pruitt and Riechert, 2009). Just as in the classical Hawk-Dove model (Maynard Smith and Price, 1973), the fitness of aggressive spiders was found to be frequency dependent: aggression carried lower fitness when there were many aggressive spiders. It is this striking resemblance to the Hawk-Dove evolutionary game that grounded our modelling approach originally. By expanding on the classical model in this chapter, we provide a compelling case that the patterns observed by Pruitt and Goodnight (2014) could have evolved by individual selection alone.

This is not to suggest that group selection had no role in the evolution of aggressiveness in *A. studiosus*, but simply that the relative importance of individual and group selection is currently unknown. Group and individual selection both offer plausible accounts that are rooted in empirical observations, so further experimentation will be necessary to determine whether group selection is the stronger force and hence whether *A. studiosus* exhibits group adaptation in accordance with Sober and Wilson's (2011) definition.

3.4.2 Multi-level selection types 1 and 2

There are two main frameworks for analysing group selection/multi-level selection problems: MLS1 and MLS2 (Section 1.1.4, this thesis; Damuth and Heisler, 1988; Okasha, 2006). Each has different consequences for the question of group adaptation, so it is important to choose the correct approach. In MLS1 we pay attention to individual fitness, and whether individual *versus* group characteristics are responsible for fitness differences in the population. In contrast, MLS2 considers the fitness of groups and individuals entirely independently, requiring a group reproduction process and the proliferation of offspring *groups*. In MLS2, individual selection and group selection need not be related at all, though individual selection within each group may change group characteristics over time (Damuth and Heisler, 1988). The MLS1 framework is generally considered more appropriate for studying the majority of group selection scenarios (Damuth and Heisler, 1988; Okasha, 2006).

Researchers have predominantly employed MLS1 when studying aggression in *A. studiosus*: Pruitt and Goodnight (2014) define group selection according to the MLS1 framework of Wade (1978) and Goodnight (2013), and Pruitt and Goodnight's (2014) critics also employ the MLS1 framework implicitly (Gardner, 2015; Smallegange and Egas, 2015; Krupp, 2016; Biernaskie and Foster, 2016). However, the empirical measurements Pruitt and Goodnight (2014) performed in their original study lend themselves more naturally to an MLS2 analysis. They measure how many offspring colonies each colony produces over two generations (rather than the fitness of the individuals in those groups, or the sizes of those offspring groups) and they evaluate fitness exclusively according to the survival or extinction of groups.

An experimental design grounded in the MLS1 framework is needed to convincingly resolve the question of group adaptation in *A. studiosus*. Unlike Pruitt and Goodnight's (2014) approach, an MLS1 experiment would enable the direct comparison of individual selection and group selection (Okasha, 2006), and therefore the evaluation of group adaptation (Sober and Wilson, 2011). Indeed, both major methods employed to evaluate MLS1 – the Price (1972) equation and contextual analysis (Heisler and Damuth, 1987; Goodnight et al., 1992) – directly quantify the contributions of individual *versus* group selection (Okasha, 2004a, 2006; Goodnight, 2013; Marshall, 2015).

3.4.3 Adaptive adjustment of colony composition

We have so far considered the initial finding that resource availability affects the relationship between colony size and aggressiveness in naturally occurring colonies, and that this size/aggressiveness relationship appears to be beneficial for group and individual fitness. Yet, the *A. studiosus* colonies deployed by Pruitt and Goodnight

(2014) also began to adapt back, over two generations, towards a size/aggressiveness combination that was more characteristic of their home site. This occurred irrespective of the site at which they were deployed so this adjustment behaviour was not responsive to immediate changes in the level of prey availability. This suggests their tendency to adjust in a particular way is likely to have evolved over many previous generations at their home sites, allowing the species to evolve distinct adjustment behaviours at high and low resource sites. The mechanism could be, for example, a tendency for aggressive spiders to disperse at a particular colony size, with the optimal number evolving differently based on the site's resource level. This and other alternative explanations were considered by Pruitt and Goodnight (2014) but were assumed to be *group* adaptations.

However, such adjustment behaviour could also have evolved by exclusively individual incentives. Dispersal in *A. studiosus* is stochastic (Grinsted et al., 2015) so spiders from a given lineage may occupy colonies of many different sizes. Yet we find the equilibrium frequency of aggressive spiders at a given resource level changes with colony size. Therefore, if a spider were able to manipulate levels of aggression (either of nest-mates, offspring, or themselves) during their lifetime according to the size of colony they were in, they would outperform a fixed strategy over multiple generations. Any ability to do so would be strongly selected for due to the positive impact on foraging success, demonstrated by the Hawk-Dove game, which is tightly linked with the reproductive fitness of individuals in spiders (Petersen, 1950; Kessler, 1971). Consequently, not only can both group and individual selection explain the tendency for colonies to be found in certain combinations of size and aggressiveness, but they can also both explain the multi-generational adjustment of colony composition.

3.4.4 Relation to previous work

Another individual-level explanation for the relationship between size and colony aggressiveness in *A. studiosus* was provided by Smallegange and Egas (2015). They developed an environmental threshold model wherein individual spider aggressiveness is a plastic trait, and a high variance in social cues is assumed. Plasticity of this kind should be unlikely due to the trait's high heritability (Pruitt and Goodnight, 2014), though this heritability estimate may have been inflated because it ignored non-genetic effects, such as the extended 4-6 week period of obligate maternal care in *A. studiosus* (Brach, 1977). In any case, our models are compatible with individual plasticity of aggressiveness and a high variance in social cues, but these are not requirements. Most importantly, however, our models predict the fitness trade-offs that this previously proposed explanation assumes (Smallegange and Egas, 2015).

The original study (Pruitt and Goodnight, 2014) has also been interpreted with an inclusive fitness framework (Gardner, 2015). A compromise of aggression between the individuals' optimum and the interests of their colony, due to modest relatedness among colony members, can also match the observations. Biernaskie and Foster (2016) take this approach much further, generating separate high and low resource models, and evaluating the fit to the data for different hypothetical functions that relate group-level aggression and group success. However, Gardner (2015) and Biernaskie and Foster (2016) assume aggressive spiders always outcompete their docile counterparts within colonies, contradicting experimental evidence from Pruitt and Riechert (2009). Both models express the negative effects of aggression only in group fitness, ignoring within-group frequency-dependent selection (Pruitt and Riechert, 2009).

Gardner (2015) and Biernaskie and Foster (2016) do explore different ways of distributing the group cost of aggression among colony members, but by failing to accurately model within-group dynamics, they enforce a compromise between group and individual incentives that may not actually exist. As previously noted, our work demonstrates that observed levels of aggression in *A. studiosus* colonies are also consistent with exclusively individual incentives. From an inclusive fitness perspective, this means that even if there were very low relatedness and consequently little or no kin selection, we may expect the same outcome, owing only to *personal*, within-group fitness. Again, the question of whether kin selection (or equivalently group selection) played a role, remains open.

Following Okasha (2015), Krupp (2016) used causal graphs to consider whether Pruitt and Goodnight's (2014) work constitutes a demonstration of group selection. He concluded that Pruitt and Goodnight (2014) failed to "ensure that [the group] phenotype directly caused group rather than individual fitness". If it caused individual fitness, and group fitness was merely an aggregation of individual fitness, an individual-level explanation would be preferable. However, fitness was demonstrated by Pruitt and Goodnight (2014) primarily in terms of the probability of group extinction. Extinction, being a group-level event, is a good candidate for a direct group fitness effect, especially when occurrences are associated with a group deficiency. Different biological factors were correlated with group extinction in high and low resource sites, and indeed both of these pointed to group-level failures (Pruitt and Goodnight, 2014). In high resource sites, extinct colonies were more likely to exhibit signs of social parasitism – heterospecific spiders in the web – presumably due to insufficient web defense. In low resource sites, extinct colonies exhibited greater rates of egg-case cannibalism, suggesting the group had failed to capture sufficient prey. Together, the evidence suggests that, contra Krupp (2016), group selection did occur. We do, however, agree that Pruitt and Goodnight (2014) failed to consider

the role of individual selection, which is, again, fundamental to evaluations of group *adaptation* (Sober and Wilson, 2011; Gardner and Grafen, 2009).

3.4.5 Conclusion

We find that *A. studiosus* colony compositions that appear to confer a group survival advantage (Pruitt and Goodnight, 2014) are also those compositions that would be expected to evolve through individual selection alone, with no between-group competition. Our findings follow from the combination of Hawk-Dove dynamics with empirical observations of prey availability in *A. studiosus* (Pruitt and Goodnight, 2014) and cooperative capture in the closely related *A. eximius* (Yip et al., 2008). By providing an individual-level explanation, we question whether group selection is predominantly responsible for the observed adaptation. The balance of power between individual and group selection is yet to be determined in this system, and until it is, *A. studiosus* does not provide sufficient evidence for group adaptation (Sober and Wilson, 2011).

In chapters 2 and 3, we have focused on colony/group-level evolution and its relation to population-level and individual-level evolution. In the final research chapter we look at evolution occurring higher up in the biological hierarchy: between whole species. By looking at the relative contributions of species-level selection and individual selection to the evolution of an organismic trait in the Solanaceae plant family, we demonstrate how two levels of selection can be intimately related despite operating across vastly different scales. Unlike the case of *A. studiosus* and eusocial lateralization before it, the following chapter incorporates evidence from disparate sources in order to combine the effects of the two levels of selection. Given present technology, and the immense scale of the study system, such a combination would be inconceivable without the use of modelling. Therefore, we highlight the power of modelling approaches not just for reinterpreting empirical evidence, but also for generating evolutionary insight that cannot be obtained through any other means.

Chapter 4

Species selection and individual selection in the Solanaceae plant family

This chapter is based on a paper under review with *Evolution* (Calcraft et al., submitted[b]). A preliminary version of this work was presented at the *European Conference on Artificial Life*, 2013, Taormina, Italy, and published in its peer-reviewed proceedings (Calcraft et al., 2013). This conference paper is appended to this thesis, Appendix B.

Abstract

The evolution of individual traits is rarely attributed to selection pressure between whole species because adaptation within species (between individuals) should be considerably faster and stronger. When there are conflicting interests, individual incentives are expected to dominate, and when interests are aligned, species selection may be hard to detect. Recently, however, species selection has been shown to favour the individual trait of self-incompatibility in one plant family, the Solanaceae, based upon molecular phylogenetic analysis. Moreover, it has been suggested that species selection may be maintaining self-incompatibility against the short-term interests of individuals. Here we combine population genetic models with a birth-death model of species selection to determine how both individual- and species-level conditions affect the maintenance of self-incompatibility in the plant family as a whole. We find that species selection can only affect the long-term evolution of self-incompatibility when population sizes are very small, or selection averages out to very weak at the individual level. By combining techniques from distinct theoretical approaches, we offer a causal, mechanistic account of trait evolution, elucidating the intersection of higher and lower level selection processes in the maintenance of outcrossed sexual reproduction.

4.1 Introduction

Species selection, the result of Darwinian competition between entire species rather than between individuals, has been proposed as a level of selection operating above individuals that can lead to the evolution and maintenance of traits (Lewontin, 1970; Stanley, 1975; Arnold and Fristrup, 1982; Jablonski, 2008). If a species' trait affects its rate of speciation or extinction (analogues of reproduction and death in individual selection), and this trait is inherited by its daughter species, the logic of natural selection should follow. However, individual-level selection is generally considered more powerful than higher selective levels such as species selection, and may prevent the expression of its effects in nature (Lewontin, 1970; Rice, 1995). This is principally because individual survival and reproduction events – which drive individual selection – are orders of magnitude more frequent than speciation and extinction events – which drive species selection.

The recent introduction of techniques based on molecular phylogenies has revolutionised the study of species selection (Jablonski, 2008; Pyron and Burbrink, 2013), though fossil records have been used to investigate it in the past (Van Valen, 1975). Because of these advances, and the resulting proliferation of species selection hypotheses, it is more important than ever to examine the assumptions underlying such approaches. Indeed, Chevin (2016) recently noted the lack of dynamical understanding in the species selection literature. If we want to go further than cataloguing statistical associations, towards understanding the causes of evolutionary change, mechanistic accounts are vital. We here investigate the evolutionary dynamics of a system that is well-studied at the population level and the species level, but is lacking an integrated approach that takes advantage of data from modern phylogenetic techniques.

The evolution and maintenance of outcrossed sexual reproduction in plants has a long history in theoretical biology, with an extensive literature exploring it at the population level (reviewed in Busch and Delph, 2012). Species selection has also been repeatedly implicated in the maintenance of sexual reproduction in both plants and animals (Van Valen, 1975; Vienne et al., 2013). At the species level, the most data-driven approach is seen in the study of self-compatibility (SC) and self-incompatibility (SI) within the Solanaceae (Goldberg et al., 2010), a plant family that includes economically-important crops such as the potato, tomato, aubergine and chili pepper. The trait of interest, self-incompatibility, promotes sexual reproduction with genetically dissimilar individuals (outcrossing) by enabling hermaphroditic plants to reject their own pollen, preventing the high levels of self-fertilisation (selfing) seen in self-compatible plants.

To investigate species selection on SI and SC within the Solanaceae, Goldberg

et al. (2010) derive a maximum likelihood model from molecular data, estimating rates of speciation and extinction for SC and SI species, as well as rates of transition between SI and SC. They find self-incompatible species to have a lower extinction rate than SC species and consequently a higher rate of diversification throughout the phylogeny (Goldberg et al. 2010; Goldberg and Igić 2012, but see Rabosky and Goldberg 2015). This lower extinction rate may be due to, for example, outcrossing's tendency to increase genetic diversity and reduce vulnerability to disease (Hurst and Peck, 1996; King and Lively, 2012). However, SI species undergo frequent transitions to SC, assumed to be the result of selection for SC at the individual level, while transitions back from SC to SI are negligibly rare. Thus, transitions are continuously promoting self-compatibility in the Solanaceae, but these lineages go extinct more frequently than the species that remain self-incompatible. The diversification advantage of self-incompatibility is significant: current phylogenetic estimates suggest approximately 34% of lineages will perpetually remain SI despite frequent breakdowns to SC (Igic et al., 2004; Goldberg et al., 2010).

At the individual level, self-fertilising, self-compatible plants produce offspring that are generally less fit than self-incompatible individuals' outcrossed offspring. This fitness penalty for selfed offspring is termed inbreeding depression, and is principally due to the expression of deleterious recessive mutations (Barrett, 1988). However, SC individuals may offset inbreeding depression with other advantages. SC individuals are able to pass on their genes with higher probability, as they can generate offspring from the fusion of their own ovules and pollen, while still exporting their genes as pollen to other outcrossing plants. They may also benefit from reproductive assurance because they are not dependent on inbound pollen when it is scarce (Maynard Smith, 1978; Lloyd, 1992; Igic et al., 2008; Busch and Delph, 2012). On the other hand, SI individuals, in addition to avoiding inbreeding depression, may accrue beneficial mutations faster through the sharing of genes (Fisher, 1930), which could allow them to adapt faster to changing environments. Consequently, there are costs and benefits to both SC and SI at both the individual and species level, though which level of selection is more important remains unclear.

Here we investigate the relative contributions of individual- and species-level selection to the evolution of SI and SC in the Solanaceae by incorporating population genetic theory into a birth-death model of species selection. Birth-death models of species selection simulate phylogenies, creating a tree of life with a branching process that uses specified rates (or probabilities) for speciation and extinction. The birth-death model used to study self-incompatibility was a binary state speciation and extinction (BiSSE) model (Maddison et al., 2007; Goldberg et al., 2010). In the BiSSE model, every species was either self-incompatible or self-compatible, and had different speciation and extinction rates accordingly. There was also some regular

rate of transition from self-incompatibility to self-compatibility. This transition rate is the critical point at which individual selection and species selection come into contact, because transitions from self-incompatibility to self-compatibility are driven by self-compatible mutants invading populations through individual selection (and/or genetic drift). Apart from the transition rate, species selection occurs entirely independently of selection at lower levels, because it is a multi-level selection type 2 (MLS2) process (Section 1.1.4, this thesis; Damuth and Heisler, 1988; Okasha, 2006) where species reproduce themselves (through speciation) orthogonally to the reproduction of individuals.

In order to model individual selection and species selection together, we first simulate underlying populations (individual selection) to predict transition rates from SI to SC. We then use these transition rates to drive a birth-death model (species selection), using the speciation and extinction rates for SI and SC species from Goldberg et al. (2010). We then calculate our outcome of interest from this birth-death model: the expected percentage of SI species that would be perpetually maintained within the Solanaceae. We are able to vary the strength of species selection in the birth-death model, as well as the selection parameters for the underlying populations that provide the transition rate. We can therefore compare how changes to individual-level selection pressure and species-level selection pressure affect the evolution of self-incompatibility in the Solanaceae. Our analysis suggests that, under almost all conditions, individual selection must average out to nearly neutral for species selection to affect the long-term maintenance of SI.

4.2 Materials and methods

We estimated the average transition rate from fully self-incompatible (SI) Solanaceae populations to full self-compatibility (SC), by stochastically simulating the arrival and spreading of SC mutations in unstructured populations. We obtained expected transition rates for many different conditions, covering the order of magnitude range of population sizes observed in the Solanaceae (Richman et al., 1996), and SC mutation rates observed in flowering plants (Lewis, 1979). We simulated many different strengths of individual selection, including positive and negative selection for SC at the individual level, and neutral selection with genetic drift only. We also simulated the invasion of SC mutants with a more naturalistic model using ecologically relevant parameters. We finally incorporated these population-level simulations into a species-level model, predicting whether SI would be maintained or lost by evolution in the long-term if transitions were to occur at the rates implied by our population simulations. We computed the long-term evolutionary outcome over many differ-

ent strengths of selection at both the individual and species level, to determine the relative contributions of these evolutionary forces.

4.2.1 Basic selection model

We constructed a Wright-Fisher process (Wright, 1931; Fisher, 1930) for a diploid population of size N_e, the effective population size, which is the number of individuals in an idealised (model) population that would yield the expected level of genetic drift in a given natural population, accounting for complications like the sex ratio and non-random mating (Kimura, 1983). Trials started with a fully SI population, all plants homozygous for the SI allele. We simulated discrete non-overlapping generations, with mutation, selection, and genetic drift. A constant fitness advantage of s (a selection coefficient) for homozygous SC over homozygous SI was employed with semi-dominance, heterozygotes having an advantage $\frac{s}{2}$. Simulations were terminated upon fixation of the SC mutant – with all plants homozygous for the SC allele – whereupon the number of generations passed was recorded. The process is highly stochastic, so the mean number of generations over 1000 Monte Carlo trials was taken for each experimental condition. The reciprocal of this mean fixation time was recorded as the average transition rate λ from SI to SC.

4.2.2 Genotypic model

We also simulated transitions from SI to SC using a more naturalistic model, allowing us to investigate the effect of important biological and ecological factors. We built upon Uyenoyama et al.'s (2001) genotypic model for the invasion of an SC mutant, incorporating the genetics of self-incompatibility in the Solanaceae and the male- and female-specific mutations that may confer self-compatibility. The model simulates reproduction differently for SI and SC individuals, with SC individuals producing some proportion of selfed offspring that avoid the two-fold cost of sex (Maynard Smith, 1978) but that are punished by a fixed level of inbreeding depression. Inbreeding depression is the reduction in fitness for self-fertilised offspring due to deleterious recessive mutations (Lloyd, 1992) that self-incompatibility avoids but that self-compatible plants are vulnerable to.

Our genotypic model tracked the evolution of simulated gene frequencies over time, specifically, the frequency of each of the three types of plant in the population: homozygous SI plants, heterozygotes, and homozygous SC plants. As in our basic selection model, the simulation started with 100% homozygous SI. At the start of each generation, the probability of each of the three types of parent plant giving rise to offspring of each of the three types was calculated: homozygous SI to heterozygous, heterozygous to homozygous SC, and so on (see Table 4.2). These

parent-offspring probabilities were then weighted by the proportion of each parent-type in the population to form a trinomial distribution (see Section 4.2.3 for detailed explanation). N_e new individuals were then generated stochastically according to this probability distribution. Each new generation was thus picked with probabilities proportional to zygotic fitness, while the random drawing provided genetic drift in the same way as the Wright-Fisher simulations of Kimura and Ohta (1969). When the population neared fixation for the SC mutant ($> 99\%$ of the population homozygous for SC, in line with Goldberg et al.'s (2010) criteria), the simulation terminated and the number of generations passed was recorded. Long-running simulations were aborted after 10^{11} generations if fixation failed to occur. Trials were again repeated 1000 times, and the reciprocal of the mean fixation time was recorded as the average transition rate λ from SI to SC. Examples of individual trials can be seen in Figure 4.1.

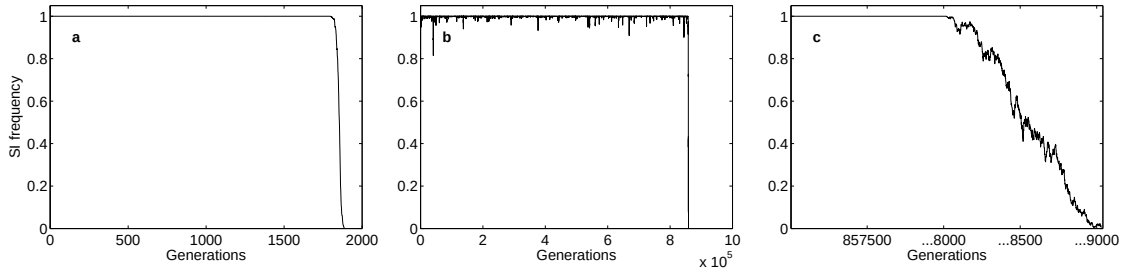

Figure 4.1: Example trial sequences illustrating typical invasions of SC mutants. (**a**) A full trial where SC has strong selective effect ($\delta = 0.3$). An SC mutant arrives and is quickly lost three times over the first 1,382 generations, then the fourth arrives at generation 1,796, and spreads to fixation by generation 1,885. (**b**) A full trial for which selection for SC is nearly neutral ($\delta = 0.65$). Many SC mutants arrive and are lost by drift, with a mutant eventually drifting to fixation after approximately 860,000 generations. (**c**) An expanded view of the nearly neutral trial (**b**), showing the successful fixation of the SC mutant over a 3,000 generation window. Trials recorded from the genotypic model, $\mu = 10^{-6}$, $N_e = 1{,}000$.

μ	Mutation rate
N_e	Effective population size
s	Fitness advantage of self-incompatible plants (basic model)
ϕ	Self-pollination rate (genotypic model)
δ	Inbreeding depression (genotypic model)

Table 4.1: Variables governing the basic and genotypic models of individual selection.

4.2.3 Parent-offspring probabilities

The dynamics of the genotypic model were governed by the parent-offspring probabilities, which are shown in Table 4.2 and derived below (Section 4.2.3). These probabilities depend principally on the levels of inbreeding depression, δ, and the probability of any plant receiving SC pollen rather than SI pollen, m_c. m_c itself depends on the population's current fraction of homozygous SI plants f_{ii}, heterozygotes f_{ic}, and homozygous SC plants f_{cc}:

$$m_c = \frac{\frac{1}{2}f_{ic} + f_{cc}}{f_{ii} + f_{ic} + f_{cc}} \tag{4.1}$$

We assume all plants export the same quantity of pollen. The rate of self-pollination for SC plants, ϕ, also changes the conditions under which SC evolves (Uyenoyama et al., 2001). Individual-level models such as Lloyd's (1992) often assume full self-pollination ($\phi = 1$) for SC plants. We here run the analysis with $\phi = 0.5$.

	Probability of offspring, weighted by fitness		
Parent	Homozygous SI	Heterozygous	Homozygous SC
Homozygous SI	$1 - m_c$	m_c	0
Heterozygous	$\frac{1}{2}(1 - m_c)(1 - \frac{\phi}{2})$	$\frac{1}{4}(2 - \delta\phi)$	$\frac{1}{2}(1 - m_c)(1 - \frac{\phi}{2}) + \frac{\phi}{4}(1 - \delta)$
Homozygous SC	0	$(1 - m_c)(1 - \phi)$	$m_c(1 - \phi) + \phi(1 - \delta)$

Table 4.2: Parent-offspring probabilities that define the evolution of the model. Each parent type (row) gives rise to each offspring type (column) with the probability specified in the (row, column) cell. These probabilities are additionally weighted by fitness, as some include the effect of inbreeding depression, so each row need not sum to one; probabilities are normalised before use. See Section 4.2.3 for explanation and derivations. δ is the level of inbreeding depression, ϕ is the self-pollination rate, and m_c is the probability of received (mate) pollen carrying the SC mutant (see Equation 4.1).

It is worth noting that mutations are not considered during a given invasion process – only when waiting for a mutant to arise do we observe the mutation rate. Including them has negligible effect on time to transition. Mutations *back* – from SC to SI – were also excluded. This is because self-incompatibility is a complex mechanism requiring coordination at many genetic loci (Franklin-Tong, 2008; Stone, 2002). There are therefore many possible nucleotide substitutions that can result in self-compatibility and selfing, so the probability of any particular locus being

mutated and subsequently substituted back to functional in one line of inheritance is negligible at the population level – an example of Dollo's law of irreversibility (Dollo, 1893).

Functionally, each pollen grain's plant of origin is identified to other plants (and itself) by the S-allele it carries. This is a genetically encoded protein sequence that varies between individuals, first discovered in the tobacco plant – a member of the Solanaceae (East and Yarnell, 1929). The number of unique S-alleles in a population, n, determines how effectively plants can identify pollen as self *versus* non-self, so Uyenoyama et al. (2001) explicitly consider this value n in their model of selection on self-compatibility. We simulate the population only in the limit of high n, equivalent to each plant having a unique S-allele. This simplification is required for computational feasibility, as we perform many-trial stochastic simulations of the entire invasion process under hundreds of conditions. For arbitrarily high n, the conditions for invasion of male (pollen-part) and female (stylar) mutants in diploid populations are equal. Our model is therefore agnostic between these two types of mutation, which are the most well understood routes to self-compatibility in the Solanaceae, yet whose relative prevalence is poorly understood (Stone, 2002). Whole genome duplication – polyploidization – may also lead to self-incompatibility, but transition is observed to occur at least three times more often by mutation within diploid populations than by polyploidization in the Solanaceae (Robertson et al., 2011), so our analysis focuses on the former.

Zygotic fitness

The probabilities of each plant type producing offspring of a given type can be stated in terms of the self-pollination rate, ϕ, and the probability that received (mate) pollen is an SC mutant, m_{c}. The model assumes the fraction of pollen received by a plant that is from itself is ϕ. In homozygous SC plants, all of this pollen then competes on equal footing with the $1-\phi$ fraction of inbound pollen for ovules to sire. Heterozygous plants, on the other hand, will reject half of their own self-pollen, as half of their pollen will carry a functional SI gene, and thereby be rejected by matching one of the SI genes expressed in the plant's style. This means the effective selfing rate for heterozygotes is $\frac{\phi}{2}$, with $1-\frac{\phi}{2}$ inbound from other plants. We assume here that the limited resource is ovules, rather than physical pollen access, so the rejected $\frac{\phi}{2}$ of self-pollen for heterozygote selfers does not take up space that could otherwise be used by non-self pollen. That is, in the competition for ovule siring, only *accepted* self-pollen shares space with inbound pollen, hence the inbound pollination rate of $1-\frac{\phi}{2}$.

Again, the probability of receiving SC pollen rather than SI pollen, m_{c}, depends

on the current composition of the population (Equation 4.1).

Homozygous SI plants (denoted ii) always reproduce by outcrossing, receiving pollen from other plants in the population. They may produce homozygous SI offspring only if they receive SI rather than SC pollen (which they do with probability $1 - m_c$):

$$\text{ii} \rightarrow \text{ii} = 1 - m_c$$

They may produce heterozygous offspring (denoted ic) by receiving SC pollen from other plants (with probability m_c), that will fuse with their SI ovules:

$$\text{ii} \rightarrow \text{ic} = m_c$$

Finally, homozygous SI plants are incapable of producing homozygous SC (cc) offspring:

$$\text{ii} \rightarrow \text{cc} = 0$$

Heterozygous plants may produce homozygous SI offspring only by outcrossing (at a rate the complement of their selfing rate: $1 - \frac{\phi}{2}$), and if their SI gene rather than their SC gene is transmitted (at a rate $\frac{1}{2}$), and if the pollen they receive is also SI (with probability $1 - m_c$).

$$\text{ic} \rightarrow \text{ii} = (1 - \tfrac{\phi}{2}) \cdot \tfrac{1}{2} \cdot (1 - m_c)$$

They may produce heterozygous offspring by: outcrossing $(1 - \frac{\phi}{2})$ with an ovule containing their SI gene $(\frac{1}{2})$ and receiving SC pollen m_c. Or by: outcrossing $(1 - \frac{\phi}{2})$ with an ovule containing their SC gene $(\frac{1}{2})$ and receiving SI pollen $(1 - m_c)$. Or by: selfing $(\frac{\phi}{2})$ with an ovule containing their SI gene $(\frac{1}{2})$ and receiving SC pollen (1; all selfed pollen contains the SC gene). This last probability is multiplied by $(1 - \delta)$, to punish offspring produced by selfing with inbreeding depression.

$$\begin{aligned}\text{ic} \rightarrow \text{ic} &= (1 - \tfrac{\phi}{2}) \cdot \tfrac{1}{2} \cdot m_\text{c} \\ &+ (1 - \tfrac{\phi}{2}) \cdot \tfrac{1}{2} \cdot (1 - m_\text{c}) \\ &+ \tfrac{\phi}{2} \cdot \tfrac{1}{2} \cdot (1 - \delta) \\ &= \tfrac{1}{4}(2 - \delta\phi)\end{aligned}$$

Heterozygotes may finally produce homozygous SC offspring by: outcrossing $(1 - \frac{\phi}{2})$ with an ovule containing their SC gene $(\frac{1}{2})$ and receiving SC pollen m_c. Or by: selfing $(\frac{\phi}{2})$ with an ovule containing their SC gene $(\frac{1}{2})$ and receiving SC pollen (1); this last probability again multiplied by $(1 - \delta)$ for inbreeding depression.

$$\begin{aligned}\text{ic} \rightarrow \text{cc} &= (1 - \tfrac{\phi}{2}) \cdot \tfrac{1}{2} \cdot (1 - m_\text{c}) \\ &+ \tfrac{\phi}{2} \cdot \tfrac{1}{2} \cdot (1 - \delta)\end{aligned}$$

Homozygous SC plants cannot produce homozygous SI offspring.

$$\text{cc} \rightarrow \text{ii} = 0$$

They may produce heterozygous offspring by outcrossing $(1 - \phi)$ and receiving SI pollen $(1 - m_\text{c})$, given that all their ovules contain SC genes.

$$\text{cc} \rightarrow \text{ic} = (1 - \phi) \cdot (1 - m_\text{c})$$

Homozygous SC plants may finally produce homozygous SC offspring by: outcrossing $(1 - \phi)$ and receiving SC pollen (m_c). Or by: selfing (ϕ); punished by inbreeding depression δ.

$$\begin{aligned}\text{cc} \rightarrow \text{cc} &= (1 - \phi) \cdot m_\text{c} \\ &+ \phi \cdot (1 - \delta)\end{aligned}$$

Probability collation

These probabilities are used to form the trinomial distribution sampled from at each generation. The selection probability for each genotype in the trinomial is formed by multiplying the offspring-generating probabilities by the relative frequency of each

type in the current generation, and then categorically summing by each offspring genotype. Before drawing from the distribution, it must be normalised so the expected output frequencies of the three types total one. This normalisation is required because we pre-multiply the reproduction penalties for inbreeding depression (δ) in the equations to account for fitness differences, making some of the possible paths less probable than others, and in turn reducing the total probability sum from one. This process of normalisation is equivalent to dividing through by mean fitness.

4.2.4 Species selection

For both our basic and genotypic models, we calculated the effect of the resulting SI-to-SC transitions on the percentage of species expected to be perpetually maintained as SI by evolution – the equilibrium percentage of SI species (Igic et al., 2004). We used a binary state speciation and extinction (BiSSE) model of phylogenetic evolution (Maddison et al., 2007; Goldberg et al., 2010; Igic et al., 2004), in which species selection favoured SI species continuously from its ancestral state (approximately 36 million years ago), at the strength observed by Goldberg et al. (2010), while being opposed by transitions from SI to SC caused by our simulated invasions of SC mutants. The model assumes an unbounded metapopulation of species with fixed rates of speciation and extinction (that differ for SI and SC species), and a fixed rate of transition from SI to SC (SC to SI transitions are omitted as they are negligibly rare (Igic et al., 2004; Goldberg et al., 2010)). The rate of transition inferred by Goldberg et al. (2010) suggests a mixed equilibrium, with SI maintained in approximately 34% of species in the Solanaceae. By considering alternative rates of transition that may result from different conditions at the individual level, we observed how the maintenance of self-incompatibility in the Solanaceae could be affected both by individual-level factors and by species selection.

The percentage of species expected to be perpetually maintained as SI by evolution, P_{SI}, was calculated using the birth-death model equilibrium (Igic et al., 2004):

$$P_{\mathrm{SI}} = 100\left(1 - \frac{\lambda}{\text{species selection multiplier} \cdot r}\right) \tag{4.2}$$

where λ is the SI-to-SC transition rate, and r is the net diversification rate advantage of SI species over SC species (0.847 per million years (Goldberg et al., 2010)):

$$\begin{aligned} r &= \text{SI speciation rate} - \text{SI extinction rate} \\ &\qquad - (\text{SC speciation rate} - \text{SC extinction rate}) \\ &= 0.847\text{E-}6. \end{aligned} \tag{4.3}$$

For our genotypic model, we additionally quantified the levels of inbreeding depression that supported the ongoing presence of both SI and SC species in the plant family, focusing on three key outcomes: equal proportions (50% SI), SI's near-complete loss (5% SI) and SI's near-complete maintenance (95% SI). The stochastic nature of the results meant we were unlikely to find the value of inbreeding depression that would give rise to a precise SI percentage of e.g. 50%, so we performed full simulations at small increments of inbreeding depression δ, and used linear interpolation to approximate the value of δ that corresponded to our key outcomes.

We further computed the equilibrium species-level percentage of SI (and the levels of inbreeding depression that corresponded to the three key outcomes) for different strengths of species selection. We considered advantages between $0.1\times$ and $10\times$ the empirically observed rate, r, by using the species selection multiplier in Equation 4.2.

λ	Transition rate from self-incompatible (SI) species to self-compatibility (SC)
r	Diversification rate advantage of SI over SC species ($= 0.847\text{E-}6$)
species selection multiplier	Value between 0.1 and 10 that modifies the relative diversification advantage of SI species over SC species

Table 4.3: Variables governing the birth-death model of species selection.

4.3 Results

4.3.1 Basic selection model

We simulated finite, unstructured populations of fully self-incompatible (SI) plants subject to mutation and constant selection pressure to determine the expected rate of arrival and fixation of self-compatible (SC) mutants, which we take as an approximation of the species-level rate of transition from SI to SC. Employing a Wright-Fisher process (Wright, 1931; Fisher, 1930), we modelled selection pressure with a selection coefficient, s, where an SC mutant plant was $(1 + s)$ times as likely to be selected for the next generation as an SI plant; negative values of s invoked selection against SC mutants that arose. We then determined the impact of these different SI-to-SC transition rates on the fate of self-incompatibility in the plant family as a whole. We incorporated our SI-to-SC transition rates into a simulated phylogeny (Goldberg

et al., 2010), in which species selection favoured SI species. From this model, we derived the percentage of species expected to remain self-incompatible over long-term evolutionary time (Equation 4.2). This allowed us to expose the effect of individual-level selection pressure on the species-level outcome.

Our simulations covered many different combinations of conditions spanning the natural ranges of mutation rates and effective population sizes for the Solanaceae (Lewis, 1979; Richman et al., 1996), and a broad range of selective pressures for and against self-compatibility at the individual level ($s = -10^{-1\ldots-5}, 0, 10^{-5\ldots-1}$).

Irrespective of the mutation rate and effective population size, increasing the selection coefficient, s, generally increases the SI-to-SC transition rate because SC mutants that arise spread faster and are less likely to be lost through drift (Figure 4.2a-c). Strong negative selection against SC ($s = -10^{-1} = -0.1$) ensures that even 100 billion generations are not enough for a single SC mutant to arise and become fixed: SC mutants are rapidly lost in favour of fitter SI individuals in the population. We terminated the simulations if 100 billion generations passed without a single invasion of an SC mutant, effectively fixing the transition rate at 10^{-5} per million years for these trials, though the actual rate may be many orders of magnitude lower. This threshold was reached by all our simulations at $s = -0.1$. With so few transitions to SC at $s = -0.1$, the expected equilibrium of SI species within the Solanaceae is close to 100% (Figure 4.2d-f). Thus, the species-level advantage of SI captured by the Goldberg et al. (2010) model dwarfs the infrequent transitions from SI to SC, and self-incompatibility is maintained throughout.

As negative selection gets weaker, transitions are able to occur frequently enough to prevent the family-wide maintenance of SI (e.g. $s = -10^{-5}$, Figure 4.2a,d). Negative selection can even predict the family-wide loss of SI in some cases (e.g. $s = -10^{-5}$, Figure 4.2c,f) when population sizes are small and mutation rates are high, and even when selection against self-compatibility is moderate (e.g. $s = -0.01$, $N_e = 100$, $\mu = 10^{-5}$). Smaller population sizes allow this to occur because random genetic drift plays a more substantial role in smaller populations, so transitions can continue to occur even against opposing selection pressure. Higher mutation rates also encourage the loss of SI because transition rates increase with mutation rates in general (mutants arise more frequently and in greater numbers), so transition rates are more likely to reach the level required to ensure the complete loss of SI over evolutionary time. When selection is neutral ($s = 0$), SI-to-SC transitions continue to occur due to random genetic drift, but at a marginally higher rate than at $s = -10^{-5}$ because there is no opposition from selection. At neutral, population size has little effect on transition rate (Figure 4.2a-c), as expected from neutral theory (Kimura, 1968). Increasing the population size means populations take longer to invade, but there are also more opportunities for mutants to arise. In the absence

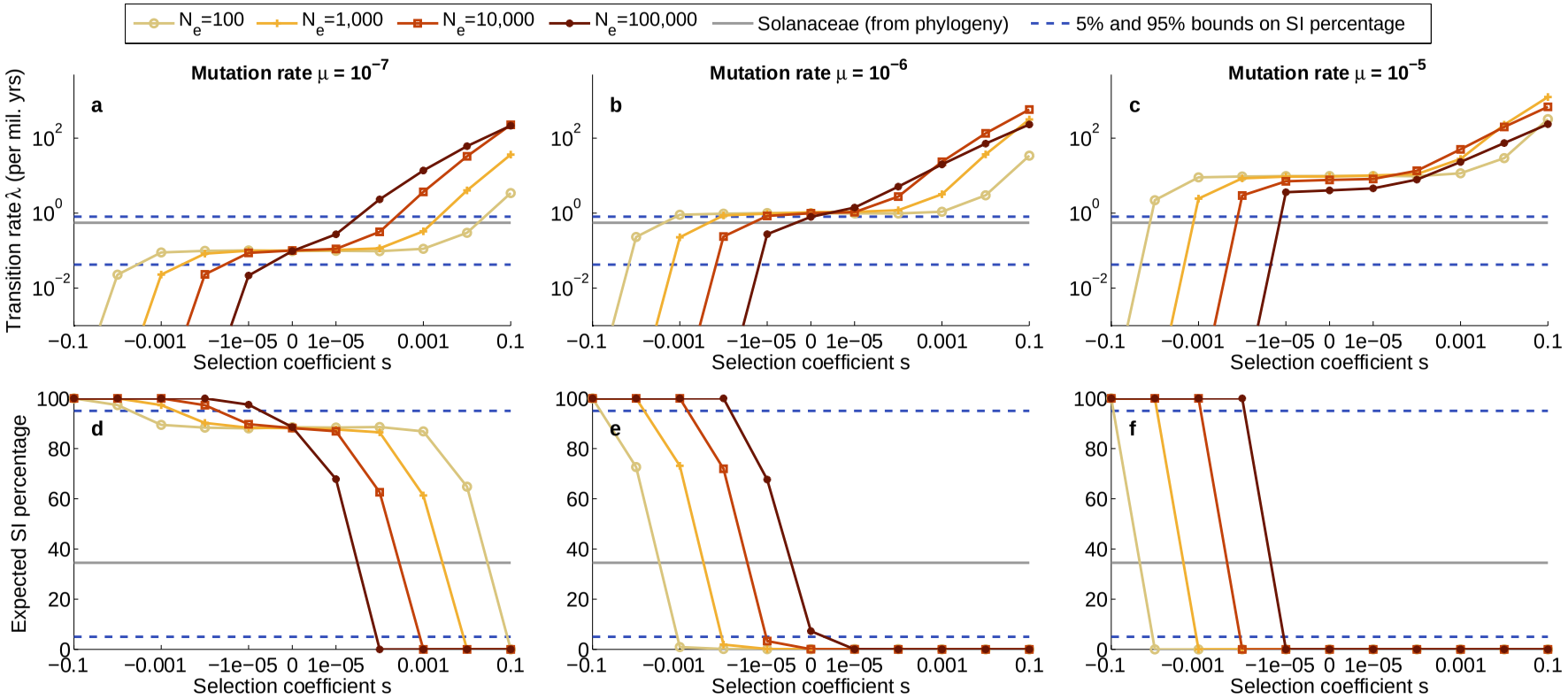

Figure 4.2: Predicted SI-to-SC transition rate λ (**a-c**; note the log scale) and expected equilibrium percentage of self-incompatible species (**d-f**) for increasing selective effect s. Equilibrium percentage assumes species selection is favouring SI (Goldberg et al., 2010) against transitions occurring at rate λ. Results shown for different effective population sizes N_e (line series) and mutation rates μ (columns). Phylogeny-based estimates for the Solanaceae (Goldberg et al., 2010) indicated by the grey horizontal line. Blue dashed lines indicate 5% and 95% boundaries for the expected SI equilibrium. Simulations exceeding 100 billion generations without a single mutant managing to invade were terminated, and are fixed at $\lambda = 10^{-5}$ per million years.

of selection, these two considerations approximately cancel out (Kimura, 1968).

Under all conditions, introducing positive selection increases the transition rate, which continues to rise as the strength of positive selection is increased (Figure 4.2a-c). Moderate positive selection for self-compatibility ($s \geq 0.01$) causes rapid fixation of SC mutants, with transition rates under almost all conditions that are orders of magnitude higher than the phylogenetic estimate made by Goldberg et al. (2010) (Figure 4.2a-c).

We calculated the transition rates that would be necessary to predict the family-wide loss of self-incompatibility (5% SI) and the family-wide maintenance of SI (95% SI) at equilibrium, assuming species selection was operating at the strength observed (Goldberg et al., 2010; Igic et al., 2004). Higher rates of transition from SI to SC reduce the expected equilibrium percentage of SI: a rate of 0.805 per lineage per million years would maintain just 5% of species within the Solanaceae as SI, even with species selection favouring SI. Transition rates above this threshold tend toward the extinction of SI. Conversely, a rate of 0.0424 transitions per lineage per million years predicts an equilibrium of 95% SI species; below this transition rate, SI tends towards complete fixation. Thus, just one order of magnitude difference in the transition rate separates these two extremes (Figure 4.2a-c). Moreover, the transition rates for moderate positive selection ($s \geq 0.01$) are not just higher than Goldberg et al.'s (2010) phylogenetic estimate, but fall well above this 5% bound: such frequent transitions to SC ensure that, even with species selection favouring SI (Goldberg et al., 2010), self-incompatibility would be destined for family-wide extinction (Figure 4.2d-f, SI = 0%).

In contrast, Goldberg et al.'s (2010) phylogenetic model predicted a mixed equilibrium with the Solanaceae retaining both SI and SC species, rather than family-wide extinction of SI. Under some conditions our results also support such a mixed equilibrium with intermediate expected SI percentages (Figure 4.2d-f). However, these conditions occur only with mild positive selection for SC ($s \leq 0.01$), neutral selection ($s = 0$), or mild negative selection against SC ($-0.01 \leq s < 0$).

As mutation rates increase (columns, left to right), so more opportunities are created for SC to invade an SI population, increasing the transition rate for all selection coefficients and effective population sizes (Figure 4.2a-c). Consequently, transition rates cross the 5% bound at lower levels of selection, s, and a greater number of conditions predict the complete loss of SI, and fixation of SC (compare Figure 4.2f with d). Even the midrange mutation rate, 10^{-6} per gamete per generation, requires mild negative selection against SC to prevent the loss of SI at all but the largest effective population size (Figure 4.2e).

Larger populations offer more opportunities for mutants to arise, causing more frequent transitions to SC under positive selection. However, this effect dimin-

ishes for combinations of high mutation rate and large population size (compare N_e = 1,000 and N_e = 10,000 in Figure 4.2b and c). Indeed, smaller population sizes can in some cases be more prone to transitions than larger ones, especially at higher mutation rates and degrees of selective pressure (compare N_e = 1,000, N_e = 10,000, and N_e = 100,000 in Figure 4.2b and c). This occurs when mutants arrive frequently enough, and with a high enough probability of fixation (due to greater selective advantage), that the average time to spread through the population becomes more important than average time for a mutation to arise. Larger populations take longer to invade and so experience lower transition rates when invasion time is significant.

In addition to their higher rates of transition, larger populations are also more sensitive to the strength of selection – both positive and negative (note the gradients, Figure 4.2a-c). This is because transitions in smaller populations are driven to a greater degree by random genetic drift, while large populations rely on selection to drive the mutation to fixation across the entire population; ten-fold changes in selection pressure can therefore impact rates of SC fixation substantially.

4.3.2 Genotypic model and inbreeding depression

Self-compatible plants confer reduced fitness upon their offspring when they self-fertilise, by exposing recessive mutations that are deleterious, or by failing to express other benefits enjoyed by hybrid offspring (heterosis) (Barrett, 1988). This fitness penalty is termed inbreeding depression, and to assess its impact on rates of transition from SI to SC, we simulated the fixation of SC mutants in populations using a one-locus genotypic model (Uyenoyama et al., 2001). The generation of offspring genotypes followed the mechanics of gametophytic self-incompatibility (Uyenoyama et al., 2001) – the form of SI employed by the Solanaceae (see *Materials and methods*). The relative fitness of SI and SC plants was determined by the level of inbreeding depression δ: the average per-progeny reduced fitness for an SC, self-fertilising parent compared with SI, outcrossing parents (Charlesworth and Charlesworth, 1987). Because the severity of inbreeding depression can vary between populations and species, and across time, we ran our simulations for many values of δ between 0 and 1.

Higher inbreeding depression δ punishes SC mutants, reducing the predicted rate of transition from SI to SC (Figure 4.3a-c). For low levels of inbreeding depression, individual selection consistently favours SC and transitions are frequent, especially at larger population sizes. Transition rates decline steadily but rapidly from $\delta = 0$ onwards before dropping sharply between $\delta = 0.6$ and $\delta = 0.7$ to fewer than 0.01 transitions every million years. This sharp drops occur near $\delta \approx 0.65$, the point

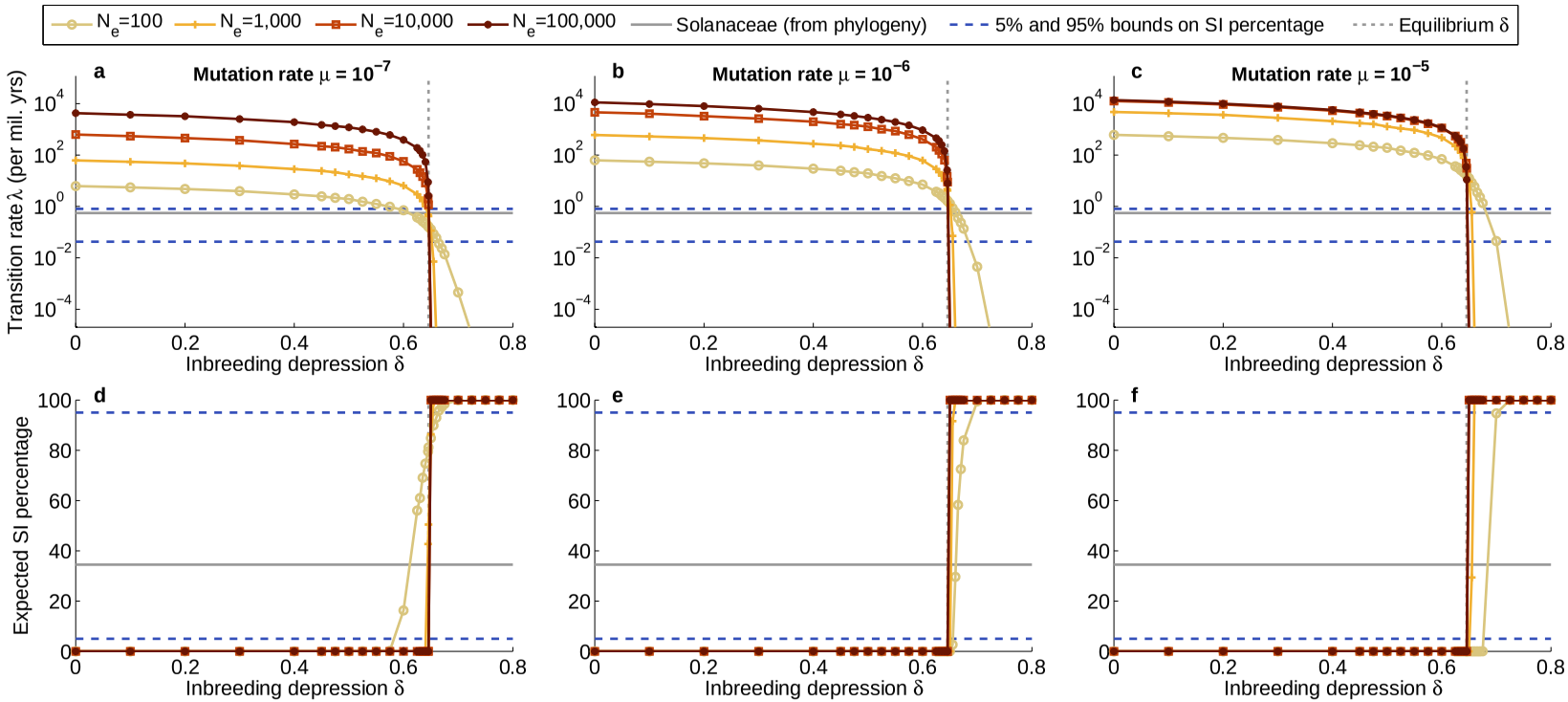

Figure 4.3: Predicted SI-to-SC transition rate λ (**a-c**; note the log scale) and expected equilibrium percentage of self-incompatible species (**d-f**) for increasing inbreeding depression δ. Equilibrium percentage assumes species selection is favouring SI (Goldberg et al., 2010) against transitions occurring at rate λ. Results shown for different effective population sizes N_e (line series) and mutation rates μ (columns). Phylogeny-based estimates for the Solanaceae (Goldberg et al., 2010) indicated by the grey horizontal line. Blue dashed lines indicate 5% and 95% boundaries for the expected equilibrium SI percentage. Dotted vertical line in grey indicates the level of δ for which selection is neutral between SI and SC (Uyenoyama et al., 2001). Simulations exceeding 100 billion generations without a single mutant managing to invade were terminated, and are fixed at $\lambda = 10^{-5}$ per million years. Note that results for $N_e = 10{,}000$ and $N_e = 100{,}000$ are near-equal in **c-f**, obscuring results for $N_e = 10{,}000$.

at which selection is neutral between SI and SC (Uyenoyama et al., 2001). At all mutation rates, larger population sizes see their sharp drop in transition rate slightly before the neutral threshold, while smaller populations drop slightly after. The descent is slowest at the smallest population size, $N_e = 100$, mirroring the observation that smaller population sizes are less reactive to changes in selection pressure (demonstrated previously, Figure 4.2), and can maintain regular transitions to SC even with moderate selection against SC (e.g. $s = -0.01$, Figure 4.2c).

The general relationships observed under constant selection (Figure 4.2) hold for all levels of inbreeding depression in the genotypic model (Figure 4.3): increasing mutation rates and population sizes both increase transition rates, shifting the curves upward (e.g. compare Figure 4.3a with Figure 4.3b). These effects are again reduced with higher mutation rates in populations of very large effective size (e.g. see overlap of $N_e = 10{,}000$ and $N_e = 100{,}000$ in Figure 4.3c).

Under all conditions in our genotypic model, there is a very narrow range of inbreeding depression for which SI is maintained at some intermediate percentage: the transition rate decreases rapidly through the 5% and 95% bounds as inbreeding depression increases. Consequently, the expected percentage of SI in the Solanaceae flips between complete loss of SI to its near complete maintenance with a small increase in average inbreeding depression (Figure 4.3d-f), though the precise value of δ at which this occurs varies with population size and mutation rate. The transition rate for the Solanaceae inferred from Goldberg et al.'s (2010) phylogeny lies between these two extremes. It intercepts our predictions at a point of steep gradient, such that very small deviations in average δ produce order of magnitude differences in the average rate of transition.

The dramatic drop in transition rate around $\delta = 0.65$ is a reflection of the sharp difference in the effect of selection *for* versus selection *against* SC on the probability of a mutant spreading throughout an entire population. If $\delta > 0.65$, fixation becomes extremely unlikely because any new mutant is selected against and promptly lost as soon as it arrives. If SC is only weakly selected against (for example $\delta = 0.66$) though, SC can still become fixed in the population through random drift but at a lower probability than if the mutant was neutral. This fixation probability diminishes rapidly as inbreeding depression increases, selecting more strongly against any mutants that arise. Consequently, transitions to SC drop rapidly as conditions move further away from positive selection. A small increase in δ from 0.66 to 0.68 reduces transition rates to near zero for all mutation rates and for all but the smallest population size. Despite this value of δ being just a few percent higher, under these conditions barely a single transition is projected to occur over the entire evolutionary history of SI in this plant family (~36 million years), emphasising the sensitivity of this parameter value.

For values of $0 \leq \delta \leq 0.6$, transition rates are orders of magnitude higher than the 5% SI level, regardless of mutation rate or population size, because of the strong positive selection for SC when inbreeding depression is low. To attain the level of transitions inferred from the phylogeny, inbreeding depression must average out to near, or slightly above, $\delta \approx 0.65$, resulting in nearly-neutral, or weak selective pressure against the evolution of SC within populations on average. These results reinforce the conclusions of our model of constant selection, and emphasise the dramatic effect of changes in individual-level selection pressure on the fate of SI at the species level.

4.3.3 Species selection

We simulated the evolution of SI and SC through a phylogeny at different strengths of species selection for SI and determined the percentage of species expected to remain SI in the Solanaceae plant family. We computed the expected percentage of SI species for all the conditions previously considered in our genotypic model – for transition rates resulting from all combinations of mutation rate, effective population size, and inbreeding depression – and over a range of species selection multipliers between 0.1 and 10 (multiples of the Goldberg et al. (2010) rate of species selection for self-incompatibility, +0.847 per million years). We then computed the values of inbreeding depression that corresponded to equal proportions of SI and SC species being maintained perpetually in the Solanaceae, for various degrees of species selection. We also calculated the levels of inbreeding depression required to push the SI percentage up to 95% and down to 5% under each condition and species selection multiplier. These calculations identify the values for inbreeding depression and species selection simultaneously that generate interesting species-level outcomes: SI near-fixed (95%), SI and SC co-existing equally (50%), and SI near-extinct (5%), illustrating the interaction of these two processes.

Species selection of strengths 0.1 to 10 times that observed by Goldberg et al. (2010) does not make any appreciable difference to the species-level outcome for larger population sizes ($N_e \geq$ 10,000, Figure 4.4). Under these conditions, the maintenance or loss of SI in the plant family as a whole is completely determined by conditions at the individual level. If inbreeding depression is above $\delta \approx 0.65$, individual selection is acting against SC mutants, and SI is maintained in all species. However, if inbreeding depression is below $\delta \approx 0.65$, individual selection favours SC mutants, and SI is lost in all species. Thus, small changes in the average level of inbreeding depression around this point determine the evolutionary outcome, and even order-of-magnitude changes in the level of species selection cannot compensate. This is because at larger population sizes there are many opportunities for mutants

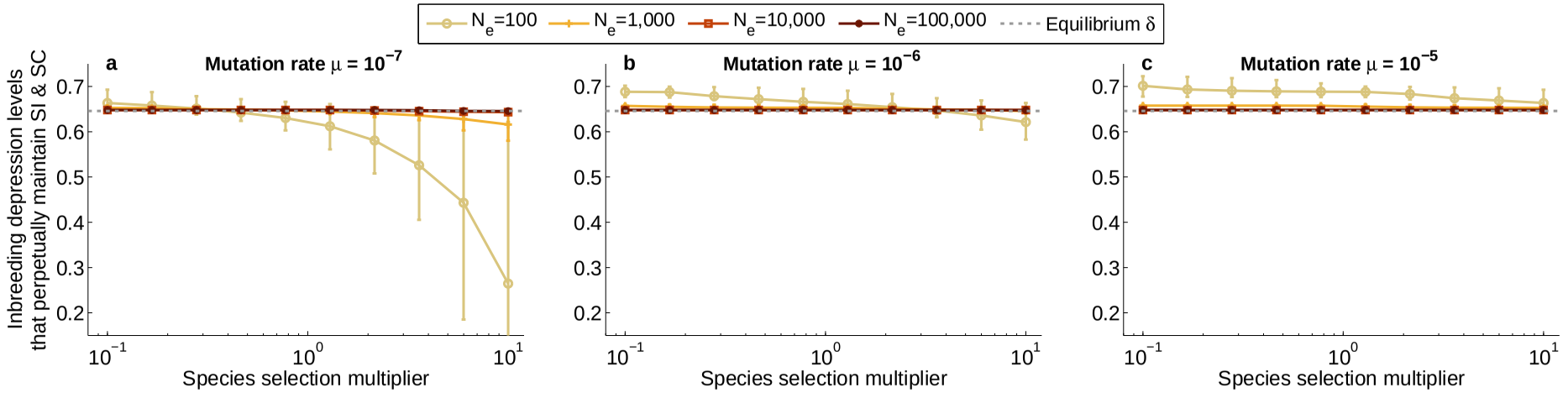

Figure 4.4: Levels of inbreeding depression required to perpetually maintain SI and SC species in the Solanaceae plant family, under various strengths of species selection. Each point indicates the level of inbreeding depression δ for which the expected SI percentage is 50%. Upper and lower error bars indicate the level at which the expected SI percentage is 95% and 5% respectively. Above the upper error bar for any given condition, SI is expected to be near-completely maintained; under the lower error bar, SI is expected to be lost in the plant family. Note that results for $N_e = 10{,}000$ and $N_e = 100{,}000$ are near-equal under all conditions, obscuring results for $N_e = 10{,}000$. Many error bars are also too small to discern from their data points. Dotted line in grey indicates the level of inbreeding depression for which selection is neutral between SI and SC (Uyenoyama et al., 2001).

to arise (because they can occur in many more individuals) and genetic drift is less significant, so the direction of even very weak selective pressure on these SC mutants can dramatically affect whether they are swiftly fixed or lost. The resulting transition rates are so low when $\delta \gtrsim 0.65$ that SI will always be maintained, regardless of any additional species-level effects, and the transition rates are so high when $\delta \lesssim 0.65$ that the complete loss of SI is largely guaranteed, even when opposed by a strong species-level advantage for SI.

For the lower effective population size of $N_e = 1{,}000$, the effects of species selection are still small. At higher mutation rates ($\mu \geq 10^{-6}$, Figure 4.4b-c), lower levels of species selection (multiplier $< 2\times$) increase the average level of inbreeding depression required to maintain SI by ~0.01. This is because a reduction in species-level pressure for SI requires more individual-level pressure for SI to compensate (and hence an increase in inbreeding depression). Species selection can exert this (albeit minor) influence at $N_e = 1{,}000$ because smaller populations are more vulnerable to drift, which can obscure the effect of weak selective forces. Thus, transition rates change less dramatically in response to the precise value of inbreeding depression when selection is nearly neutral. This diminished response keeps transition rates within a few orders of magnitude of the species selection effect size, allowing it to exert some influence, rather than the outcome depending only on the direction of individual-level selection.

The effect of species selection on $N_e = 1{,}000$ is most pronounced at high species selection multipliers and low mutation rate (multiplier $> 4\times$, $\mu = 10^{-7}$, Figure 4.4a), where the level of inbreeding depression required to maintain equal proportions of SI and SC species drops below neutral by ~0.02. That is, some substantial percentage of SI can be maintained even if selection is slightly in favour of SC at the individual level. The importance of genetic drift at smaller population sizes is again responsible: when drift is stronger, it means that mild selection for SC (at lower levels of inbreeding depression) does not automatically ensure the rapid fixation of SC mutants. Instead, the drop in transition rate between selection for SC and selection against SC is much less pronounced ($N_e = 1{,}000$, Figure 4.3a). Higher levels of species selection can therefore help to maintain SI, even when individual selection marginally favours SC. This effect only appears at the lowest order mutation rate, $\mu = 10^{-7}$, because this is where SI-to-SC transition rates are generally lower (as there are fewer mutants arising to invade), so the introduction of weak selection for SC does not immediately push transition rates so high as to preclude any opposing influence from species selection.

Species selection exerts its greatest influence at the smallest population size, $N_e = 100$. For the lowest mutation rate, $\mu = 10^{-7}$, species selection multipliers $\geq 0.8\times$ begin to radically reduce the level of inbreeding depression required to

maintain equal proportions of SI and SC species. With a 10-fold increase in the strength of species selection compared to that calculated by Goldberg et al. (2010), inbreeding depression can fall below 0.3 and still maintain at least half of all species as SI perpetually, despite strong selection for SC mutants. This is an exaggerated form of the effect seen at $N_e = 1{,}000$, where low mutation rate slows transitions, and drift (which is even more powerful at $N_e = 100$) softens the impact of selection. These two effects ensure transitions occur at a moderate rate over a broader range of inbreeding depression, not just when selection is very close to neutral. These are the conditions under which species selection can exert a significant influence because transitions occur neither overwhelmingly frequently nor overwhelmingly rarely.

As mutation rates increase ($\mu \geq 10^{-6}$, Figure 4.4b-c), the level of inbreeding depression required to maintain equal proportions of SI and SC species increases for all species selection multipliers. This is because populations are easier to invade when there are more mutants, so there needs to be stronger selection against arising SC mutants to maintain the same species-level outcome. This occurs most visibly at $N_e = 100$ again due to the dampening effect of drift, where mild negative selection against SC does not so dramatically reduce transition rates as it does at larger population sizes, which in turn allows the shift in transition rate due to mutation rate to be emphasised. Species selection is able to exert some influence at these higher mutation rates too, where higher species selection multipliers again reduce the level of inbreeding depression required to maintain both SI and SC. However, species selection is much less significant than at the lowest mutation rate, $\mu = 10^{-7}$, where it is effective over a broad range of inbreeding depression: $0.2 < \delta < 0.7$. At the midrange mutation rate, $\mu = 10^{-6}$, species selection is only effective when $0.6 < \delta < 0.7$, and then by the highest order mutation rate, $\mu = 10^{-5}$, its window of influence narrows to $0.65 < \delta < 0.7$.

4.4 Discussion

We investigated the relative importance of species and individual selection in the maintenance of self-incompatibility (SI) in the Solanaceae plant family. We find that species selection can only affect whether SI is ultimately maintained or lost when individual selection averages out to nearly neutral, or when populations are very small ($N_e = 100$) and mutation rates are low ($\mu = 10^{-7}$, the lowest of the observed range for self-compatible (SC) mutations in flowering plants (Lewis, 1979)). In all other conditions, individual selection overwhelms species selection over the long run: very small shifts in average inbreeding depression determine whether SI is lost or maintained in the whole plant family. This is the case even when simu-

lated species selection is ten-fold stronger than the level inferred from phylogenetic reconstruction (Goldberg et al., 2010).

While simulations of constant selective pressure identified the conditions most sensitive to individual selection, our genotypic model made the extent of this sensitivity clear. Simulations that incorporated the genetics of self-incompatibility showed that the level of inbreeding depression need only change by a few percent to generate transition rates orders of magnitude higher or lower than those inferred from the phylogeny. This sensitivity manifests reliably under almost all conditions in the natural range, suggesting that any elaborations of the model (such as the simultaneous evolution of inbreeding depression with selfing (Lande and Schemske, 1985)) would need to produce a sizeable compensatory mechanism to change our results qualitatively.

4.4.1 Population-level variation

The considerable impact of small deviations in average population-level factors on the maintenance of SI in the plant family as a whole implies a tight link between evolution at the species and individual levels. These population-level factors – the effective population size and the level of inbreeding depression – are expected to vary over time and between species, determined largely by local circumstances. Because the SI-to-SC transition rate is predicted to be highly dependent upon the precise values of these population parameters, population-level variation may play an exaggerated role in the Solanaceae.

Acute sensitivity to variation is probable because the phylogenetically-inferred transition rate falls within the most sensitive parameter region, where transition rates respond most dramatically to changes in inbreeding depression (Figure 4.3a-c). Therefore, species within the Solanaceae are predicted to experience epochs of high and low vulnerability to invasion by SC mutants as conditions fluctuate. Transitions to SC occur when environmental conditions favour them at the individual level, while SI lineages remain SI by not experiencing conditions favourable to SC for long enough. Consequently, the transition rate for the plant family is the mean over time (~36 million years (Goldberg et al., 2010)) of a transition rate distribution, summarising the share of time spent by SI lineages in conditions prone to, versus resistant to, SC invasion. The maintenance of self-incompatibility, then, is critically dependent on the distribution of local circumstances and attendant levels of individual selection pressure.

4.4.2 Species selection *versus* individual selection

Our combined model, incorporating population genetics into a birth-death model of phylogenetic evolution, showed that species selection was unable to influence the long-term evolution of SI under most conditions: individual selection was simply too powerful. However, a substantial species-level effect was observable at the lowest population size ($N_e = 100$) and mutation rate ($\mu = 10^{-7}$). This is because very small populations are more vulnerable to random genetic drift, and less sensitive to the direction of selection, so individual selection does not overwhelm higher levels of selection. Populations of smaller effective size may more closely resemble the conditions that bring about cladogenetic transitions to self-compatibility, where transitions occur simultaneously with speciation events. Cladogenetic transitions to SC often occur when a smaller fringe population becomes isolated and vulnerable to SC invasion, and then develops into a separate species (Goldberg and Igić, 2012). Cladogenetic transitions appear to be more common than anagenetic transitions (where SC invades an entire species, absent any isolation or speciation event) in the Solanaceae (Goldberg and Igić, 2012), which may emphasise smaller population sizes in the analysis. However, even at the smallest effective population size we modelled, species selection only exerts a strong influence (relative to individual-level selection) at the lowest mutation rate.

4.4.3 Limitations

Following previous theoretical investigations of species selection (Slatkin, 1981; Rice, 1995; Chevin, 2016), our analysis assumes species can be represented by single, unstructured populations. This simplification is to some extent mitigated by our use of empirical measurements for the effective population size in the Solanaceae (Richman et al., 1996), and by covering the full range of observed effective sizes in our analysis. We also assume species compete with one another in an unbounded metapopulation, and that speciation and extinction rates are constant. As Chevin (2016) notes, density dependence, the influence of unmeasured traits, and environmental variation can all potentially violate these assumptions. Unfortunately, there is insufficient data available to confidently include these features in the analysis, or indeed to simulate population structures more accurately.

4.4.4 Related work

Recent theoretical work found that, when looking at macroevolutionary patterns, randomness in speciation and extinction events may play a much stronger role than randomness at the population level in the form of genetic drift (Chevin, 2016).

However, our simulations show that the strength of genetic drift within species can still have a substantial impact on rates of transition in the Solanaceae. This is probably due to directional bias in the mutation rate; self-incompatibility is easy to break down (Franklin-Tong, 2008) but very unlikely to originate or be restored from a self-compatible line. This mutational bias means genetic drift is not random with respect to within-species evolution – drift is much more likely to promote SC than SI – so the strength of drift can exert more influence on within-species dynamics, with considerable macroevolutionary consequences.

4.4.5 Conclusion

Empirical examinations of species selection do not typically consider the dynamics of within-species and between-species selection simultaneously. Theoretical approaches similar to ours have been developed before (Slatkin, 1981; Rice, 1995; Chevin, 2016), but only deployed in the abstract to make claims about species selection in general. By applying this approach to the evolution of self-incompatibility in the Solanaceae, we were able to generate a causal account of selection on multiple levels. We revealed how dependent the previously observed species selection effect is on individual-level conditions, and tied these findings to measurable properties of Solanaceae populations (effective size, inbreeding depression, SC mutation rate). Our models suggest that unless population sizes are very small, species selection can only exert an influence if the average selective effect at the individual level is close to neutral. Therefore, the fate of self-incompatibility in the Solanaceae is determined by the strength of both individual- and species-level selection. If the phylogenetically-inferred transition rate (Goldberg et al., 2010) is accurate (see Rabosky and Goldberg, 2015), our analysis suggests SI's fate in the Solanaceae could easily have been determined exclusively by individual selection, but conditions have in fact transpired to allow species selection to be a relevant force.

Chapter 5

Discussion

Group behaviour is an evolutionary innovation that pervades the tree of life, and is fundamental to the human condition. Its particular manifestations have perplexed researchers at least since the time of Darwin (1859), and neither its evolutionary origins nor its ongoing maintenance are particularly well understood. Group selection and multi-level selection theories hold a central place in the scientific debate around these issues, attempting to explain how altruistic behaviour can evolve, and how group characteristics are shaped by evolution.

In this thesis, I applied theoretical tools to multi-level biological systems that had already received empirical attention. In doing so, I generated new insights and understanding, as well as predictions that can be tested through further empirical work. Thus, I approached matters of contemporary debate in the group selection controversy, but in a more concrete form.

Below I summarise the key contributions of the individual chapters of the thesis, and then draw general conclusions about research on the levels of selection, touching on the value of modelling and its relationship with empirical work.

5.1 Summary

In Chapter 2, a closer examination of population- and colony-level dynamics revealed that, despite reliable lateralization at the colony level, population-level lateralization is predicted not to exist in eusocial colonies where predators/competitors interact with multiple colonies in their lifetime. This finding contradicts existing theory, which predicts that highly social species (such as those that form eusocial colonies) should have strong population-level lateralization. However, the absence of population-level lateralization is nevertheless consistent with the empirical evidence published on eusocial insect lateralization, because the methods used have been unable to distinguish between colony-level and population-level lateralization (by, for

instance, not tracking which colonies individuals were from or sampling a very small number of colonies). Additionally, Chapter 2 developed a first-principles model of predator/competitor learning, and shows how this model is consistent with new empirical research on *Formica rufa* (colonies are ~60% lateralized, Appendix A) over a range of parameters. The results suggest that, where there is some approximately linear benefit to self-consistency (here, within-colony coordination), an intermediate level of self-consistency may be optimal in order to balance this benefit with the adaptive advantage of confusing predators and competitors.

In Chapter 3, a game theoretic analysis of a group-living, sub-social spider species, *Anelosimus studiosus*, demonstrated that patterns of colony aggression could be explained with individual incentives alone, ignoring any potential group-level effects on fitness. In contrast, Pruitt and Goodnight (2014) claimed their experimental work on colony aggression demonstrated group-level *adaptation*. However, according to Sober and Wilson's (2011) definition of group adaptation, this would require the evidence to suggest group-level effects are key to the evolution of colony aggression, and more important than individual incentives. Sober and Wilson (2011) argue that if a trait is to be termed a group adaptation, group selection should be the dominant force in its evolution. Further, there is near-consensus among theorists that within-group and between-group effects should be compared when evaluating group selection (Eldakar and Wilson, 2011; Okasha, 2004b; Gardner and Grafen, 2009). Such a comparison is crucially missing from Pruitt and Goodnight's (2014) study, and the model presented in this thesis demonstrates the importance of this omission when trying to understand the causes of evolution. The presentation of an alternative, game-theoretic explanation shows that the patterns of colony aggression in *A. studiosus* have multiple possible explanations. Further empirical work is required to determine whether group selection or individual selection is more powerful in this system, and therefore whether group adaptation has occurred in this sub-social spider.

Chapter 4 investigated species selection through the development of a model that incorporated population genetic theory into a birth-death phylogenetic model of species evolution. Species selection, which is a form of group selection that has received relatively limited theoretical attention, has seen a marked increase in empirical work since the late 2000s. The model presented here demonstrated the concurrent mechanics of species and individual selection by applying natural selection theory at two levels simultaneously in a concrete biological system – the Solanaceae plant family. Additionally, the model offered insights into the average conditions of Solanaceae populations over evolutionary time by working backwards from species-level empirical data. The conditions under which species selection is able – and importantly, unable – to affect the traits of organisms has here been demonstrated with particular

reference to this well-studied family of plants, strengthening the empirical relevance of the model.

5.2 Conclusions

While the immediate aim of each research chapter is to contribute to the field's understanding of their respective biological scenarios, together they demonstrate a number of principles that may inform future work on the levels of selection.

Multi-level selection scenarios need to be evaluated on a case-by-case basis

An accurate causal account of evolution is critical to generate reliable predictions and understanding that can be generalised. The causal story of evolution is not complete without considering (the potential for) natural selection on all relevant levels in the biological hierarchy. This is first demonstrated by the evolution of lateralization. Previous theory considered only individual-level and population-level lateralization (Ghirlanda and Vallortigara, 2004) so the discovery of colony-level lateralization was surprising (Appendix A). By modelling colonies explicitly, it became clear that natural selection would not operate as previously predicted. Indeed, according to the model, colony-level lateralization is not only possible, but likely. Furthermore, population-level lateralization may itself be undermined by between-colony competition when predators/competitors interact with multiple colonies. This is because between-colony competition can generate frequency-dependent selection at the colony level that continually drives population-level lateralization towards zero – an effect that could not have been anticipated by looking only at the individual and population level.

In the case of the sub-social spider, *Anelosimus studiosus*, investigating the expected effects of natural selection within colonies, rather than only between colonies as Pruitt and Goodnight (2014) did in their original study, revealed that group selection is not the only explanation for differences in colony aggression between high and low resource sites. By paying attention to individual selection as well as group selection, the strong claim of their study – that they have demonstrated group adaptation – can be refuted, highlighting the need for further evidence to support their claim. Without considering both levels of selection in this scenario, Pruitt and Goodnight (2014) were led to the erroneous conclusion that they had very strong evidence that group selection was the driver of evolution in this scenario, whereas actually they had shown only that some degree of group selection was in effect.

While a multi-level selection analysis of self-compatibility in the Solanaceae plant

family did not overturn the study's main result (Goldberg et al., 2010), it did generate new insight into the interdependence of species selection and individual selection. Indeed, it built upon Goldberg et al.'s (2010) work by demonstrating how their surprising finding – that species selection is affecting the evolution of an individual character trait – was possible. Again, by considering both levels of selection together, a more comprehensive understanding was achieved. Species selection does not act alone. Instead, if it is to exert any evolutionary influence, the strength of lower-level processes need to fall within a particular range.

Models are especially valuable for studying multi-level selection

Modelling approaches can be particularly powerful in this research area because evidence is rarely gathered at multiple levels simultaneously, so the interactions of multiple levels of selection may need to be inferred theoretically. This point is particularly relevant for the Solanaceae, where no conceivable experiment (at least with current technology) could gather data on individual-level selection and species-level selection simultaneously. The levels of selection in this scenario are no less intertwined; it is merely much harder to gather the appropriate evidence than from, for example, subdivided microbial populations. It is nevertheless necessary for a causal understanding of evolution that we identify the strength of the selection process at each level and how selection at one level can preclude or enable selection at another. For now, modelling is the only way to achieve this type of understanding in biological systems that span such extents in time and space.

Even in systems where the study of a single population would be sufficient to make reasonable inferences, such as colonies of the red wood ant, *Formica rufa*, or sub-social spider, *Anelosimus studiosus*, obtaining multi-level evidence is extremely challenging. Especially so when attempting simultaneous measurement. Consequently, modelling can be a tremendous asset, allowing researchers to integrate separate lines of evidence that pertain to different levels of the biological hierarchy. Models of this nature need not provide a complete answer to the evolutionary question to be valuable. Merely identifying the strength of selection required at a particular level, or how fitness needs to be distributed among phenotypes to match one hypothesis rather than another, can help direct future experiments and develop the field's understanding of the problem.

Concrete models, grounded in empirical work, are particularly important

In evolutionary biology and behavioural ecology, theory and empirical work are not very well integrated. Conferences, for example, tend to be either theory-driven or largely experimental. There may be some posters and talks that cross over at the fringes, but there are relatively few researchers that speak both languages and appreciate both approaches. This may shift as experimentalists increasingly rely on complex statistical techniques, and in some cases modelling, to interpret their results. However, at present, collaboration remains rarer than desired (Haller, 2014). Of course, both sides have something to learn from the other. Much modelling work can be criticised for being too abstract, or for relying on assumptions that an empiricist could not countenance. Likewise, without a thorough grasp of the underlying theory, experimentalists may over-interpret their data or design experiments that do not effectively distinguish between relevant hypotheses.

This problem does not have a simple solution. But from a modelling perspective, applying theory to concrete examples has definite advantages and moves the field, however gradually, in the right direction. It hopefully encourages the modeller to be stricter about their assumptions and adherence to reality. General, abstract models are certainly appealing, but biological details can have a profound impact on results. Biology is an inherently messy medium for research, so highly general theories should perhaps be more of an occasional aspiration than an absolute requirement. More importantly, however, modelling concrete biological scenarios with existing empirical interest increases the likelihood that theoretical work will be read by, and accessible to, experimentalists. Historians of biology note that the revolutionary work of Fisher (1930), Wright (1931) and Haldane (1932) in founding population genetics did not reach general acceptance, and certainly not general application, until the publication of works such as *Evolution: the new synthesis* by Huxley (1942), which bridged the explanatory gap (Bowler, 2003). Clearly, each individual contribution to biological problem solving made by theory will not have an impact anywhere near that of the modern evolutionary synthesis. Nevertheless, this type of communication is critical to the advancement of science at any level – both in validating and correcting the assumptions made by theorists, and in unlocking the value of theoretical insight for wider applications in biology.

Glossary

altruism Altruistic actions benefit other organisms while incurring a cost to the actor. In this thesis, we adopt an intuitive definition of altruism that evaluates the costs and benefits proximately, at the time the action is performed (preferred by Trivers (1971), Wilson (1992), Wilson and Sober (1998), Fletcher and Zwick (2006), and Silk (2013)), rather than ultimately, over evolutionary time (preferred by e.g. Marshall (2011a)). An action may be ultimately selfish because it tends to maximise personal fitness in the long-term, but is altruistic (on our account) if it involves the immediate donation of benefit to others at a cost to oneself.

frequency-dependent selection A scenario where the selective advantage of some character trait depends on the prevalence (or frequency) of that character trait in the population or group (Strickberger, 2000).

lateralization The localisation or preference of function on one side of an organism, such as the human tendency to have a dominant left or right hand (Hardyck and Petrinovich, 1977; Rogers, 2000; McManus, 2004).

MLS1 Multi-level selection type 1. A process of evolution occurring at multiple levels where the focus is on the fitness of lower level units. The higher level (e.g. groups of organisms) may contribute to evolution by affecting the fitness of the lower level units (e.g. individual organisms), but the fitness of the higher level entities are only an aggregate of the fitness of its lower level constituent units (Damuth and Heisler, 1988; Okasha, 2006).

MLS2 Multi-level selection type 2. A process of evolution occurring at multiple levels where the levels are considered distinctly, and have separate, incommensurable evolutionary processes and notions of fitness. A key requirement for MLS2 is that the higher level units must have a reproductive process of their own, such as speciation events in species selection (Damuth and Heisler, 1988; Okasha, 2006).

relatedness The degree of relatedness, r, between an individual A and B is the probability that A and B share a given gene above the baseline probability of a random individual in the population having that gene (Hamilton, 1970, 1972; Grafen, 1984). A value of $r = 0$ implies A and B are no more likely to share a given gene than A is with any individual in the population, while a value of $r = 1$ implies A and B have identical genetic makeup.

species selection The differential speciation and extinction of species owing to properties of those species that are inherited by daughter species (Stanley, 1975; Vrba, 1984). Species selection is an example of an MLS2 process (Okasha, 2006).

Bibliography

Abbot, P., J. Abe, J. Alcock, S. Alizon, J. A. Alpedrinha, M. Andersson, J.-B. Andre, M. Van Baalen, F. Balloux, S. Balshine, et al. (2011). 'Inclusive fitness theory and eusociality'. In: *Nature* 471.7339, E1–E4.

Achterberg, K. van and J. M. Durán (2011). 'Oviposition behaviour of four ant parasitoids (Hymenoptera, Braconidae, Euphorinae, Neoneurini and Ichneumonidae, Hybrizontinae), with the description of three new European species'. In: *Zookeys* 125, pp. 59–106.

Allee, W. C., O. Park, A. E. Emerson, T. Park, K. P. Schmidt, et al. (1949). *Principles of Animal Ecology*. Philadelphia PA, USA: WB Saundere Co. Ltd.

Allen, B., M. A. Nowak, and E. O. Wilson (2013). 'Limitations of inclusive fitness'. In: *Proceedings of the National Academy of Sciences of the United States of America* 110.50, pp. 20135–20139.

Anfora, G., E. Frasnelli, B. Maccagnani, L. J. Rogers, and G. Vallortigara (2010). 'Behavioural and electrophysiological lateralization in a social (*Apis mellifera*) but not in a non-social (*Osmia cornuta*) species of bee'. In: *Behavioural Brain Research* 206.2, pp. 236–239.

Arnold, A. J. and K. Fristrup (1982). 'The theory of evolution by natural selection: a hierarchical expansion'. In: *Paleobiology* 8.02, pp. 113–129.

Barrett, S. C. H. (1988). 'The evolution, maintenance, and loss of self-incompatibility systems'. In: *Plant Reproductive Ecology: Patterns and Strategies*. Oxford, UK: Oxford University Press, pp. 98–124.

Bell, A. (submitted). 'On the lateralized motor behaviour of insects'. PhD thesis. University of Sussex.

Bell, A. T. and J. E. Niven (2014). 'Individual-level, context-dependent handedness in the desert locust'. In: *Current Biology* 24.10, R382–R383.

Bertram, B. C. R. (1978). 'Living in groups: predators and prey'. In: *Behavioural Ecology: An Evolutionary Approach*. Oxford, UK: Blackwell Scientific Publications, pp. 64–96.

Biernaskie, J. M. and K. R. Foster (2016). 'Ecology and multilevel selection explain aggression in spider colonies'. In: *Ecology Letters* 19.8, pp. 873–879.

Birch, J. and S. Okasha (2015). 'Kin selection and its critics'. In: *Bioscience* 65.1, pp. 22–32.

Blount, Z. D., C. Z. Borland, and R. E. Lenski (2008). 'Historical contingency and the evolution of a key innovation in an experimental population of *Escherichia coli*'. In: *Proceedings of the National Academy of Sciences of the United States of America* 105.23, pp. 7899–7906.

Boomsma, J. J., M. Beekman, C. K. Cornwallis, A. S. Griffin, L. Holman, W. O. H. Hughes, L. Keller, B. P. Oldroyd, and F. L. W. Ratnieks (2011). 'Only full-sibling families evolved eusociality'. In: *Nature* 471.7339, E4–E5.

Bourke, A. F. (2011a). *Principles of Social Evolution*. Oxford, UK: Oxford University Press.

— (2011b). 'The validity and value of inclusive fitness theory'. In: *Proceedings of the Royal Society of London B: Biological Sciences* 278, pp. 3313–3320.

Bowler, P. J. (2003). *Evolution: The History of an Idea*. Oakland CA, USA: University of California Press.

Brach, V. (1977). '*Anelosimus studiosus* (Araneae: Theridiidae) and the evolution of quasisociality in theridiid spiders'. In: *Evolution* 31.1, pp. 154–161.

Brian, M. V. (1983). *Social Insects: Ecology and Behavioural Biology*. London, UK and New York NY, USA: Chapman and Hall.

Burke, M. K. and M. R. Rose (2009). 'Experimental evolution with *Drosophila*'. In: *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* 296.6, R1847–R1854.

Busch, J. and L. Delph (2012). 'The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization'. In: *Annals of Botany* 109.3, pp. 553–562.

Calcraft, P., P. Husbands, and A. Philippides (2013). 'An environmental model of self-compatibility transitions in the Solanaceae plant family'. In: *Advances in Artificial Life, ECAL*. Vol. 12. Cambridge MA, USA: MIT Press, pp. 1107–1113.

Calcraft, P., P. Husbands, A. Philippides, and J. Niven (submitted[a]). 'Frequency-dependent individual selection can explain group composition in sub-social spiders'. In: *Ecology & Evolution*.

Calcraft, P., P. Husbands, J. Niven, and A. Philippides (submitted[b]). 'Species selection and individual selection in the Solanaceae plant family'. In: *Evolution*.

Cashmore, L., N. Uomini, and A. Chapelain (2008). 'The evolution of handedness in humans and great apes: a review and current issues'. In: *Journal of Anthropological Sciences* 86, pp. 7–35.

Charlesworth, D and B Charlesworth (1987). 'Inbreeding depression and its evolutionary consequences'. In: *Annual Review of Ecology and Systematics* 18, pp. 237–268.

Chevin, L.-M. (2016). 'Species selection and random drift in macroevolution'. In: *Evolution* 70, pp. 513–525.

Collins, R. L. and S. Glick (1985). 'On the inheritance of direction and degree of asymmetry'. In: *Cerebral Lateralization in Nonhuman Species*. London, UK: Academic Press, pp. 41–71.

Cusumano, A., E. Peri, and S. Colazza (2016). 'Interspecific competition/facilitation among insect parasitoids'. In: *Current Opinion in Insect Science* 14, pp. 12–16.

Damuth, J. and I. L. Heisler (1988). 'Alternative formulations of multilevel selection'. In: *Biology and Philosophy* 3.4, pp. 407–430.

Darwin, C. (1859). *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life*. London, UK: John Murray.

— (1871). *The Descent of Man, and Selection in Relation to Sex*. London, UK: John Murray.

Darwin, C. and A. Wallace (1858). 'On the tendency of species to form varieties; and on the perpetuation of varieties and species by natural means of selection'. In: *Journal of the Proceedings of the Linnean Society of London: Zoology* 3.9, pp. 45–62.

Dawkins, R. (1976). *The Selfish Gene*. Oxford, UK: Oxford University Press.

— (1982). *The Extended Phenotype*. Oxford, UK: Oxford University Press.

Denenberg, V. H. (1981). 'Hemispheric laterality in animals and the effects of early experience'. In: *Behavioral and Brain Sciences* 4.01, pp. 1–21.

Di Paolo, E. A., J. Noble, and S. Bullock (2000). 'Simulation models as opaque thought experiments'. In: *Artificial Life VII: The Seventh International Conference on the Simulation and Synthesis of Living Systems*, pp. 497–506.

Dollo, L. (1893). 'Les lois de l'évolution'. In: *Bulletin de la Société Beige de Géologie de Paléontologie et d'Hydrologie* 7, pp. 164–166.

Duda, R. O., P. E. Hart, and D. G. Stork (2012). *Pattern Classification*. New York NY, USA: Wiley-Interscience.

East, E. and S. Yarnell (1929). 'Studies on self-sterility. VIII. Self-sterility allelomorphs'. In: *Genetics* 14.5, pp. 455–487.

Eldakar, O. T. and D. S. Wilson (2011). 'Eight criticisms not to make about group selection'. In: *Evolution* 65.6, pp. 1523–1526.

Eshel, I. and L. L. Cavalli-Sforza (1982). 'Assortment of encounters and evolution of cooperativeness'. In: *Proceedings of the National Academy of Sciences of the United States of America* 79.4, pp. 1331–1335.

Ferriere, R. and R. E. Michod (2011). 'Inclusive fitness in evolution'. In: *Nature* 471.7339, E6–E8.

Fisher, R. A. (1930). *The Genetical Theory of Natural Selection*. Oxford, UK: Clarendon Press.

Fletcher, J. A. and M. Zwick (2006). 'Unifying the theories of inclusive fitness and reciprocal altruism'. In: *The American Naturalist* 168.2, pp. 252–262.

Foster, K. R., T. Wenseleers, and F. L. Ratnieks (2006). 'Kin selection is the key to altruism'. In: *Trends in Ecology & Evolution* 21.2, pp. 57–60.

Foster, K. (2009). 'A defense of sociobiology'. In: *Cold Spring Harbor Symposia on Quantitative Biology*. Vol. 74. Cold Spring Harbor Laboratory Press, pp. 403–418.

Franklin-Tong, V. E. (2008). *Self-incompatibility in Flowering Plants: Evolution, Diversity, and Mechanisms*. Berlin and Heidelberg, Germany: Springer-Verlag.

Frasnelli, E, G Vallortigara, and L. Rogers (2011). 'Right-left antennal asymmetry of odour memory recall in three species of Australian stingless bees'. In: *Behavioural Brain Research* 224.1, pp. 121–127.

Frasnelli, E. (2013). 'Brain and behavioral lateralization in invertebrates'. In: *Frontiers in Psychology* 4.939, pp. 1–10.

Frasnelli, E., I. Iakovlev, and Z. Reznikova (2012a). 'Asymmetry in antennal contacts during trophallaxis in ants'. In: *Behavioural Brain Research* 232.1, pp. 7–12.

Frasnelli, E., G. Vallortigara, and L. J. Rogers (2012b). 'Left–right asymmetries of behaviour and nervous system in invertebrates'. In: *Neuroscience & Biobehavioral Reviews* 36.4, pp. 1273–1291.

Frasnelli, E., A. Haase, E. Rigosi, G. Anfora, L. J. Rogers, and G. Vallortigara (2014). 'The bee as a model to investigate brain and behavioural asymmetries'. In: *Insects* 5.1, pp. 120–138.

Frost, G. T. (1980). 'Tool behavior and the origins of laterality'. In: *Journal of Human Evolution* 9.6, pp. 447–459.

Furey, R. E. (1998). 'Two cooperatively social populations of the theridiid spider *Anelosimus studiosus* in a temperate region'. In: *Animal Behaviour* 55.3, pp. 727–735.

Gardner, A. (2015). 'Group selection versus group adaptation'. In: *Nature* 524.7566, E3–E4.

Gardner, A. and A. Grafen (2009). 'Capturing the superorganism: a formal theory of group adaptation'. In: *Journal of Evolutionary Biology* 22.4, pp. 659–671.

Gardner, A., S. A. West, and G. Wild (2011). 'The genetical theory of kin selection'. In: *Journal of Evolutionary Biology* 24.5, pp. 1020–1043.

Ghirlanda, S. and G. Vallortigara (2004). 'The evolution of brain lateralization: a game-theoretical analysis of population structure'. In: *Proceedings of the Royal Society of London B: Biological Sciences* 271.1541, pp. 853–858.

Ghirlanda, S., E. Frasnelli, and G. Vallortigara (2009). 'Intraspecific competition and coordination in the evolution of lateralization'. In: *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 364.1519, pp. 861–866.

Godfray, H. C. J. (1994). *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton NJ, USA: Princeton University Press.

Goldberg, E. E. and B. Igić (2012). 'Tempo and mode in plant breeding system evolution'. In: *Evolution* 66.12, pp. 3701–3709.

Goldberg, E. E., J. R. Kohn, R. Lande, K. A. Robertson, S. A. Smith, and B. Igić (2010). 'Species selection maintains self-incompatibility'. In: *Science* 330.6003, pp. 493–495.

Goodnight, C. (2013). 'On multilevel selection and kin selection: contextual analysis meets direct fitness'. In: *Evolution* 67.6, pp. 1539–1548.

Goodnight, C. J., J. M. Schwartz, and L. Stevens (1992). 'Contextual analysis of models of group selection, soft selection, hard selection, and the evolution of altruism'. In: *The American Naturalist*, pp. 743–761.

Grafen, A. (1984). 'Natural selection, kin selection and group selection'. In: *Behavioural Ecology: An Evolutionary Approach*. Vol. 2. Oxford, UK: Wiley-Blackwell, pp. 62–84.

Grinsted, L., T. Bilde, and J. D. Gilbert (2015). 'Questioning evidence of group selection in spiders'. In: *Nature* 524.7566, E1–E3.

Gromko, M. H. (1977). 'What is frequency-dependent selection?' In: *Evolution* 31.2, pp. 438–442.

Haldane, J. B. S. (1932). *The Causes of Evolution*. 36. Princeton NJ, USA: Princeton University Press.

— (1955). 'Population genetics'. In: *New Biology* 18, pp. 34–51.

Haller, B. C. (2014). 'Theoretical and empirical perspectives in ecology and evolution: a survey'. In: *Bioscience* 61, pp. 1–10.

Hamilton, W. D. (1963). 'The evolution of altruistic behavior'. In: *The American Naturalist* 97.896, pp. 354–356.

— (1964a). 'The genetical evolution of social behaviour. I'. In: *Journal of Theoretical Biology* 7.1, pp. 1–16.

— (1964b). 'The genetical evolution of social behaviour. II'. In: *Journal of Theoretical Biology* 7.1, pp. 17–52.

— (1970). 'Selfish and spiteful behaviour in an evolutionary model'. In: *Nature* 228.5277, pp. 1218–1220.

— (1972). 'Altruism and related phenomena, mainly in social insects'. In: *Annual Review of Ecology and Systematics* 3.1, pp. 193–232.

— (1975). 'Innate social aptitudes of man: an approach from evolutionary genetics'. In: *Biosocial Anthropology* 53, pp. 133–55.

Hardyck, C. and L. F. Petrinovich (1977). 'Left-handedness'. In: *Psychological Bulletin* 84.3, pp. 385–404.

Hassell, M. P. (1978). *The Dynamics of Arthropod Predator-Prey Systems*. Princeton NJ, USA: Princeton University Press.

Heino, M., J. A. Metz, and V. Kaitala (1998). 'The enigma of frequency-dependent selection'. In: *Trends in Ecology & Evolution* 13.9, pp. 367–370.

Heinze, J. (2010). 'Conflict and conflict resolution in social insects'. In: *Animal Behaviour: Evolution and Mechanisms*. Berlin and Heidelberg, Germany: Springer-Verlag, pp. 151–178.

Heisler, I. L. and J. Damuth (1987). 'A method for analyzing selection in hierarchically structured populations'. In: *The American Naturalist* 130.4, pp. 582–602.

Herre, E. A. and W. T. Wcislo (2011). 'In defence of inclusive fitness theory'. In: *Nature* 471.7339, E8–E9.

Heuts, B. A., P. Cornelissen, and D. Y. M. Lambrechts (2003). 'Different attack modes of Formica species in interspecific one-on-one combats with other ants (Hymenoptera: Formicidae)'. In: *Annales Zoologici* 53.2, pp. 205–216.

Hillis, W. D. (1993). 'Why physicists like models and why biologists should'. In: *Current Biology* 3.2, pp. 79–81.

Holbrook, C. T., C. M. Wright, and J. N. Pruitt (2014). 'Individual differences in personality and behavioural plasticity facilitate division of labour in social spider colonies'. In: *Animal Behaviour* 97, pp. 177–183.

Hölldobler, B. and E. O. Wilson (2009). *The Superorganism: The Beauty, Elegance, and Strangeness of Insect Societies*. New York NY, USA: WW Norton & Company.

Hopkins, W. D., M. J. Wesley, M. K. Izard, M. Hook, and S. J. Schapiro (2004). 'Chimpanzees (*Pan troglodytes*) are predominantly right-handed: replication in three populations of apes.' In: *Behavioral Neuroscience* 118.3, pp. 659–663.

Hughes, W. O., B. P. Oldroyd, M. Beekman, and F. L. Ratnieks (2008). 'Ancestral monogamy shows kin selection is key to the evolution of eusociality'. In: *Science* 320.5880, pp. 1213–1216.

Hunt, E. R., T. O'Shea-Wheller, G. F. Albery, T. H. Bridger, M. Gumn, and N. R. Franks (2014). 'Ants show a leftward turning bias when exploring unknown nest sites'. In: *Biology Letters* 10.12.

Hurst, L. D. and J. R. Peck (1996). 'Recent advances in understanding of the evolution and maintenance of sex'. In: *Trends in Ecology & Evolution* 11.2, pp. 46–52.

Huxley, J. (1966). 'A discussion on ritualization of behaviour in animals and man – Introduction'. In: *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 251.772, pp. 249–271.

Huxley, J. et al. (1942). *Evolution: The Modern Synthesis*. New York NY, USA: Harper & Brothers.

Igic, B., L. Bohs, and J. R. Kohn (2004). 'Historical inferences from the self-incompatibility locus'. In: *New Phytologist* 161.1, pp. 97–105.

Igic, B., R. Lande, and J. R. Kohn (2008). 'Loss of self-incompatibility and its evolutionary consequences'. In: *International Journal of Plant Sciences* 169.1, pp. 93–104.

Ivens, A. B. (2014). 'Cooperation and conflict in ant (Hymenoptera: Formicidae) farming mutualisms – A review'. In: *Myrmecological News* 21, pp. 19–36.

Jablonski, D. (1987). 'Heritability at the species level: analysis of geographic ranges of Cretaceous mollusks'. In: *Science* 238.4825, pp. 360–363.

— (2008). 'Species selection: theory and data'. In: *Annual Review of Ecology Evolution and Systematics* 39, pp. 501–524.

Johnson, J. B. and K. S. Omland (2004). 'Model selection in ecology and evolution'. In: *Trends in Ecology & Evolution* 19.2, pp. 101–108.

Josse, G. and N. Tzourio-Mazoyer (2004). 'Hemispheric specialization for language'. In: *Brain Research Reviews* 44.1, pp. 1–12.

Kessler, A (1971). 'Relation between egg production and food consumption in species of the genus *Pardosa* (Lycosidae, Araneae) under experimental conditions of food-abundance and food-shortage'. In: *Oecologia* 8.1, pp. 93–109.

Kimura, M. (1968). 'Evolutionary rate at the molecular level'. In: *Nature* 217.5129, pp. 624–626.

— (1983). *The Neutral Theory of Molecular Evolution*. Cambridge, UK: Cambridge University Press.

Kimura, M. and T. Ohta (1969). 'The average number of generations until fixation of a mutant gene in a finite population'. In: *Genetics* 61.3, pp. 763–771.

King, K. C. and C. M. Lively (2012). 'Does genetic diversity limit disease spread in natural host populations?' In: *Heredity* 109.4, pp. 199–203.

Kramer, J. and J. Meunier (2016). 'Kin and multilevel selection in social evolution: a never-ending controversy?' In: *F1000research* 5.776.

Krupp, D. (2016). 'Causality and the levels of selection'. In: *Trends in Ecology & Evolution* 31.4, pp. 255–257.

Lande, R. and D. W. Schemske (1985). 'The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models'. In: *Evolution* 39, pp. 24–40.

Lenoir, L. (2002). 'Can wood ants distinguish between good and bad food patches on the forest floor?' In: *European Journal of Soil Biology* 38.1, pp. 97–102.

Letzkus, P., W. A. Ribi, J. T. Wood, H. Zhu, S.-W. Zhang, and M. V. Srinivasan (2006). 'Lateralization of olfaction in the honeybee *Apis mellifera*'. In: *Current Biology* 16.14, pp. 1471–1476.

Levy, J. (1977). 'The mammalian brain and the adaptive advantage of cerebral asymmetry'. In: *Annals of the New York Academy of Sciences* 299.1, pp. 264–272.

Lewis, D. (1979). *Sexual Incompatibility in Plants: The Institute of Biology's Studies in Biology 110*. London, UK: Edward Arnold Ltd.

Lewontin, R. C. (1970). 'The units of selection'. In: *Annual Review of Ecology and Systematics* 1, pp. 1–18.

Liao, X., S. Rong, and D. C. Queller (2015). 'Relatedness, conflict, and the evolution of eusociality'. In: *PLoS Biology* 13.3, e1002098.

Lippolis, G., A. Bisazza, L. J. Rogers, and G. Vallortigara (2002). 'Lateralisation of predator avoidance responses in three species of toads'. In: *Laterality: Asymmetries of Body, Brain and Cognition* 7.2, pp. 163–183.

Lloyd, D. G. (1992). 'Self-and cross-fertilization in plants. II. The selection of self-fertilization'. In: *International Journal of Plant Sciences* 153.3, pp. 370–380.

Lorenz, K. (2002). *On Aggression*. London, UK: Routledge.

Luck, R. F., H. Podoler, and R. Kfir (1982). 'Host selection and egg allocation behaviour by *Aphytis melinus* and *A. lingnanensis*: comparison of two facultatively gregarious parasitoids'. In: *Ecological Entomology* 7.4, pp. 397–408.

Maddison, W. P., P. E. Midford, and S. P. Otto (2007). 'Estimating a binary character's effect on speciation and extinction'. In: *Systems Biology* 56.5, pp. 701–710.

Marshall, J. A. (2011a). 'Group selection and kin selection: formally equivalent approaches'. In: *Trends in Ecology & Evolution* 26.7, pp. 325–332.

— (2011b). 'Ultimate causes and the evolution of altruism'. In: *Behavioral Ecology and Sociobiology* 65.3, pp. 503–512.

— (2015). *Social Evolution and Inclusive Fitness Theory: An Introduction*. Oxford, UK: Princeton University Press.

— (2016). 'What is inclusive fitness theory, and what is it for?' In: *Current Opinion in Behavioral Sciences* 12, pp. 103–108.

Marshall, J. C. (1973). 'Some problems and paradoxes associated with recent accounts of hemispheric specialization'. In: *Neuropsychologia* 11.4, pp. 463–470.

Maynard Smith, J. (1964). 'Group selection and kin selection'. In: *Nature* 201, pp. 1145–1147.

— (1976). 'Group selection'. In: *The Quarterly Review of Biology* 51.2, pp. 277–283.

— (1978). *The Evolution of Sex*. Cambridge, UK: Cambridge University Press.

— (1982). *Evolution and the Theory of Games*. Cambridge, UK: Cambridge University Press.

— (1987). 'How to model evolution'. In: *The Latest on the Best: Essays on Evolution and Optimality*. Ed. by J. Dupré. Cambridge MA, USA: MIT Press, pp. 119–131.

Maynard Smith, J. and G. R. Price (1973). 'The logic of animal conflict'. In: *Nature* 246, p. 15.

McManus, C. (2004). *Right Hand, Left Hand: The Origins of Asymmetry in Brains, Bodies, Atoms and Cultures*. Cambridge MA, USA: Harvard University Press.

Mendel, G (1866). 'Versuche uber pflanzen-hybriden'. In: *Verhandlungen Des Naturforschenden Vereins Brünn* IV.

Michod, R. E. (1982). 'The theory of kin selection'. In: *Annual Review of Ecology and Systematics* 13, pp. 23–55.

Mowry, T. M., J. L. Spencer, J. E. Keller, and J. R. Miller (1989). 'Onion fly (*Delia antiqua*) egg depositional behaviour: pinpointing host acceptance by an insect herbivore'. In: *Journal of Insect Physiology* 35.4, pp. 331–339.

Myerson, R. B. (1991). *Game Theory: Analysis of Conflict*. Cambridge MA, USA: Harvard University Press.

Novgorodova, T. (2015). 'Interaction of ants with aphid enemies: Do inexperienced ants specializing in honeydew collection recognize aphidophages at their first contact?' In: *Entomological Review* 95.9, pp. 1182–1190.

Nowak, M. A. and B. Allen (2015). 'Inclusive fitness theorizing invokes phenomena that are not relevant for the evolution of eusociality'. In: *PLoS Biology* 13.4, e1002134.

Nowak, M. A., C. E. Tarnita, and E. O. Wilson (2010). 'The evolution of eusociality'. In: *Nature* 466.7310, pp. 1057–1062.

— (2011). 'Nowak et al. reply'. In: *Nature* 471.7339, E9–E10.

Okasha, S. (2004a). 'Multi-level selection, covariance and contextual analysis'. In: *The British Journal for the Philosophy of Science* 55.3, pp. 481–504.

— (2004b). 'Multilevel selection and the partitioning of covariance: a comparison of three approaches'. In: *Evolution* 58.3, pp. 486–494.

— (2006). *Evolution and the Levels of Selection*. Oxford, UK: Clarendon Press.

— (2015). 'The relation between kin and multilevel selection: an approach using causal graphs'. In: *The British Journal for the Philosophy of Science* 67.2, pp. 435–470.

Okasha, S. and C. Paternotte (2012). 'Group adaptation, formal Darwinism and contextual analysis'. In: *Journal of Evolutionary Biology* 25.6, pp. 1127–1139.

Papaj, D. R. (1993). 'Automatic behavior and the evolution of instinct: lessons from learning in parasitoids'. In: *Insect Learning: Ecology and Evolutinary Perspectives*. Dordrecht, Netherlands: Springer Science+Business Media, pp. 243–272.

Pepper, J. W. and B. B. Smuts (2002). 'A mechanism for the evolution of altruism among nonkin: positive assortment through environmental feedback'. In: *The American Naturalist* 160.2, pp. 205–213.

Petersen, B. (1950). 'The relation between size of mother and number of eggs and young in some spiders and its significance for the evolution of size'. In: *Experientia* 6.3, pp. 96–98.

Popper, K (1963). *Conjectures and Refutations*. London, UK: Routledge and Kegan Paul.

— (1993). *Unended Quest: An Intellectual Autobiography*. London, UK: Routledge.

Popper, K. (1978). 'Natural selection and the emergence of mind'. In: *Dialectica* 32.3-4, pp. 339–355.

Price, G. R. (1970). 'Selection and covariance'. In: *Nature* 227, pp. 520–521.

— (1972). 'Extension of covariance selection mathematics'. In: *Annals of Human Genetics* 35.4, pp. 485–490.

Pruitt, J. N. and C. J. Goodnight (2014). 'Site-specific group selection drives locally adapted group compositions'. In: *Nature* 514.7522, pp. 359–362.

— (2015). 'Pruitt & Goodnight reply'. In: *Nature* 524.7566, E4–E5.

Pruitt, J. N. and S. E. Riechert (2009). 'Frequency-dependent success of cheaters during foraging bouts might limit their spread within colonies of a socially polymorphic spider'. In: *Evolution* 63.11, pp. 2966–2973.

— (2010). 'How within-group behavioural variation and task efficiency enhance fitness in a social group'. In: *Proceedings of the Royal Society of London B: Biological Sciences* 278.1709, pp. 1209–1215.

Pruitt, J. N., S. E. Riechert, and T. C. Jones (2008). 'Behavioural syndromes and their fitness consequences in a socially polymorphic spider, *Anelosimus studiosus*'. In: *Animal Behaviour* 76.3, pp. 871–879.

Pyron, R. A. and F. T. Burbrink (2013). 'Phylogenetic estimates of speciation and extinction rates for testing ecological and evolutionary hypotheses'. In: *Trends in Ecology & Evolution* 28.12, pp. 729–736.

Queller, D. C. (1992). 'Quantitative genetics, inclusive fitness, and group selection'. In: *The American Naturalist* 139.3, pp. 540–558.

— (2016). 'The theory of inclusive fitness'. In: *The Quarterly Review of Biology* 91.3, pp. 343–347.

Queller, D. C., S. Rong, and X. Liao (2015). 'Some agreement on kin selection and eusociality?' In: *PLoS Biology* 13.4, e1002133.

Rabosky, D. L. and E. E. Goldberg (2015). 'Model inadequacy and mistaken inferences of trait-dependent speciation'. In: *Systems Biology* 64.2, pp. 340–355.

Ratnieks, F. L. (1988). 'Reproductive harmony via mutual policing by workers in eusocial Hymenoptera'. In: *The American Naturalist* 132.2, pp. 217–236.

Ratnieks, F. L. and P. K. Visscher (1989). 'Worker policing in the honeybee'. In: *Nature* 342.6251, pp. 796–797.

Ratnieks, F. L., K. R. Foster, and T. Wenseleers (2006). 'Conflict resolution in insect societies'. In: *Annual Review of Entomology* 51, pp. 581–608.

Raymond, M., D. Pontier, A.-B. Dufour, and A. P. Moller (1996). 'Frequency-dependent maintenance of left handedness in humans'. In: *Proceedings of the Royal Society of London B: Biological Sciences* 263.1377, pp. 1627–1633.

Rice, S. H. (1995). 'A genetical theory of species selection'. In: *Journal of Theoretical Biology* 177.3, pp. 237–245.

Richman, A. D., M. K. Uyenoyama, and J. R. Kohn (1996). 'Allelic diversity and gene genealogy at the self-incompatibility locus in the Solanaceae'. In: *Science* 273.5279, pp. 1212–1216.

Riechert, S. and T. Jones (2008). 'Phenotypic variation in the behaviour of the spider, *Anelosimus studiosus*, facilitates shift from single female to multiple female nests in colder environments'. In: *Animal Behaviour* 75, pp. 1893–1902.

Robertson, K., E. E. Goldberg, and B. Igić (2011). 'Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae'. In: *Evolution* 65.1, pp. 139–155.

Rogers, D. (1972). 'The ichneumon wasp *Venturia canescens*: oviposition and avoidance of superparasitism'. In: *Entomologia Experimentalis et Applicata* 15.2, pp. 190–194.

Rogers, L. J. (2000). 'Evolution of hemispheric specialization: advantages and disadvantages'. In: *Brain and Language* 73.2, pp. 236–253.

— (2014). 'Asymmetry of brain and behavior in animals: its development, function, and human relevance'. In: *Genesis* 52.6, pp. 555–571.

Rogers, L. J., G. Vallortigara, and R. J. Andrew (2013). *Divided Brains: The Biology and Behaviour of Brain Asymmetries*. Cambridge, UK: Cambridge University Press.

Rousset, F. and S Lion (2011). 'Much ado about nothing: Nowak et al.'s charge against inclusive fitness theory'. In: *Journal of Evolutionary Biology* 24.6, pp. 1386–1392.

Rutherford, F. and A. Ahlgren (1991). *Science for All Americans*. Oxford, UK: Oxford University Press.

Schmid-Hempel, P. (1998). *Parasites in Social Insects*. Princeton NJ, USA: Princeton University Press.

Seeley, T. D. (1989). 'The honey bee colony as a superorganism'. In: *American Scientist* 77.6, pp. 546–553.

— (1997). 'Honey bee colonies are group-level adaptive units'. In: *The American Naturalist* 150.S1, S22–S41.

Servedio, M. R., Y. Brandvain, S. Dhole, C. L. Fitzpatrick, E. E. Goldberg, C. A. Stern, J. Van Cleve, and D. J. Yeh (2014). 'Not just a theory—the utility of mathematical models in evolutionary biology'. In: *PLoS Biology* 12.12, e1002017.

Sih, A., A. M. Bell, J. C. Johnson, and R. E. Ziemba (2004). 'Behavioral syndromes: an integrative overview'. In: *The Quarterly Review of Biology* 79.3, pp. 241–277.

Silk, J. B. (2013). 'Reciprocal altruism'. In: *Current Biology* 23.18, R827–R828.

Skinner, G. (1980). 'Territory, trail structure and activity patterns in the wood-ant, *Formica rufa* (Hymenoptera: Formicidae) in limestone woodland in north-west England'. In: *Journal of Animal Ecology* 49.2, pp. 381–394.

Slatkin, M. (1981). 'A diffusion model of species selection'. In: *Paleobiology* 7.4, pp. 421–425.

Smallegange, I. M. and M. Egas (2015). 'Good for the group? Explaining apparent group-level adaptation'. In: *Trends in Ecology & Evolution* 30.7, pp. 379–381.

Sober, E. and D. S. Wilson (2011). 'Adaptation and natural selection revisited'. In: *Journal of Evolutionary Biology* 24.2, pp. 462–468.

Stanley, S. M. (1975). 'A theory of evolution above the species level'. In: *Proceedings of the National Academy of Sciences of the United States of America* 72.2, pp. 646–650.

Stone, J. (2002). 'Molecular mechanisms underlying the breakdown of gametophytic self-incompatibility'. In: *The Quarterly Review of Biology* 77.1, pp. 17–32.

Strassmann, J. E., R. E. Page Jr., G. E. Robinson, and T. D. Seeley (2011). 'Kin selection and eusociality'. In: *Nature* 471.7339, E5–E6.

Strickberger, M. (2000). *Evolution (3rd Edition)*. Boston MA, USA: Jones and Bartlett Publishers.

Trivers, R. L. (1971). 'The evolution of reciprocal altruism'. In: *The Quarterly Review of Biology* 46.1, pp. 35–57.

Turchin, P. (2013). *Complex Population Dynamics: A Theoretical/Empirical Synthesis*. Vol. 35. Princeton NJ, USA: Princeton University Press.

Uetz, G. W. (1989). 'The "ricochet effect" and prey capture in colonial spiders'. In: *Oecologia* 81.2, pp. 154–159.

Uyenoyama, M. K., Y. Zhang, and E. Newbigin (2001). 'On the origin of self-incompatibility haplotypes: transition through self-compatible intermediates'. In: *Genetics* 157.4, pp. 1805–1817.

Vallortigara, G. and L. J. Rogers (2005). 'Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization'. In: *Behavioral and Brain Sciences* 28.4, pp. 575–588.

Van Valen, L. (1975). 'Group selection, sex, and fossils'. In: *Evolution* 29, pp. 87–94.

van Veelen, M. (2009). 'Group selection, kin selection, altruism and cooperation: when inclusive fitness is right and when it can be wrong'. In: *Journal of Theoretical Biology* 259.3, pp. 589–600.

van Veelen, M., J. García, M. W. Sabelis, and M. Egas (2012). 'Group selection and inclusive fitness are *not* equivalent; the Price equation vs. models and statistics'. In: *Journal of Theoretical Biology* 299, pp. 64–80.

van Veelen, M., S. Luo, and B. Simon (2014). 'A simple model of group selection that cannot be analyzed with inclusive fitness'. In: *Journal of Theoretical Biology* 360, pp. 279–289.

Vienne, D. M. de, T. Giraud, and P.-H. Gouyon (2013). 'Lineage selection and the maintenance of sex'. In: *PLoS One* 8.6, e66906.

Völkl, W. (2001). 'Parasitoid learning during interactions with ants: how to deal with an aggressive antagonist'. In: *Behavioral Ecology and Sociobiology* 49.2-3, pp. 135–144.

Von Neumann, J. and O. Morgenstern (1944). *Theory of Games and Economic Behavior*. Princeton NJ, USA: Princeton University Press.

Vrba, E. S. (1984). 'What is species selection?' In: *Systematic Zoology* 33.3, pp. 318–328.

Wade, M. J. (1978). 'A critical review of the models of group selection'. In: *The Quarterly Review of Biology* 53.2, pp. 101–114.

Weisser, W. W., A. I. Houston, and W. Völkl (1994). 'Foraging strategies in solitary parasitoids: the trade-off between female and offspring mortality risks'. In: *Evolutionary Ecology* 8.6, pp. 587–597.

West, S. A., A. S. Griffin, and A. Gardner (2007). 'Social semantics: altruism, cooperation, mutualism, strong reciprocity and group selection'. In: *Journal of Evolutionary Biology* 20.2, pp. 415–432.

— (2008). 'Social semantics: how useful has group selection been?' In: *Journal of Evolutionary Biology* 21.1, pp. 374–385.

Wheeler, W. (1911). 'The ant-colony as an organism'. In: *Journal of Morphology* 22, pp. 307–325.

Williams, G. C. (1966). *Adaptation and Natural Selection*. Princeton NJ, USA: Princeton University Press.

Wilson, D. S. (1975). 'A theory of group selection'. In: *Proceedings of the National Academy of Sciences of the United States of America* 72.1, pp. 143–146.

— (1977). 'Structured demes and the evolution of group-advantageous traits'. In: *The American Naturalist* 111, pp. 157–185.

— (1980). *The Natural Selection of Populations and Communities*. Menlo Park CA, USA: Benjamin/Cummings Pub. Co.

Wilson, D. S. (1989). 'Levels of selection: An alternative to individualism in biology and the human sciences'. In: *Social Networks* 11.3, pp. 257–272.

— (1992). 'On the relationship between evolutionary and psychological definitions of altruism and selfishness'. In: *Biology and Philosophy* 7.1, pp. 61–68.

— (2002). *Darwin's Cathedral: Evolution, Religion, and the Nature of Society*. Chicago IL, USA: University of Chicago Press.

— (2008). 'Social semantics: toward a genuine pluralism in the study of social behaviour'. In: *Journal of Evolutionary Biology* 21.1, pp. 368–373.

Wilson, D. S. and E. Sober (1994). 'Reintroducing group selection to the human behavioral sciences'. In: *Behavioral and Brain Sciences* 17.4, pp. 585–607.

— (1998). *Unto Others: The Evolution and Psychology of Unselfish Behavior*. Cambridge MA, USA: Harvard University Press.

Wilson, D. S. and E. O. Wilson (2007). 'Rethinking the theoretical foundation of sociobiology'. In: *The Quarterly Review of Biology* 82.4, pp. 327–348.

Wilson, E. O. (1971). *The Insect Societies*. Cambridge MA, USA: Harvard University Press.

Wolf, M. and F. J. Weissing (2012). 'Animal personalities: consequences for ecology and evolution'. In: *Trends in Ecology & Evolution* 27.8, pp. 452–461.

Wright, C. M., C. T. Holbrook, and J. N. Pruitt (2014). 'Animal personality aligns task specialization and task proficiency in a spider society'. In: *Proceedings of the National Academy of Sciences of the United States of America* 111.26, pp. 9533–9537.

Wright, S. (1931). 'Evolution in Mendelian populations'. In: *Genetics* 16.2, pp. 97–159.

Wynne-Edwards, V. C. (1962). *Animal Dispersion in Relation to Social Behaviour*. Edinburgh and London, UK: Oliver & Boyd.

Yip, E. C., K. S. Powers, and L. Avilés (2008). 'Cooperative capture of large prey solves scaling challenge faced by spider societies'. In: *Proceedings of the National Academy of Sciences of the United States of America* 105.33, pp. 11818–11822.

Appendix A

Colony-level lateralization in *Formica rufa*

Chapter 3, 'Colony-level, context-dependent forelimb preference in the red wood ant, *Formica rufa*', of Adrian Bell's PhD thesis (A. Bell (submitted). 'On the lateralized motor behaviour of insects'. PhD thesis. University of Sussex) provides empirical evidence for colony-level lateralization in the red wood ant. Below is the chapter abstract, followed by the relevant results and tabular data (Table A.1).

Abstract

Lateralisation in limb control has been well-documented across a wide range of vertebrate taxa, including humans who possess population-level lateralisation thought to have evolved in response to social living. In contrast to vertebrates, there has been relatively little research on lateralisation in invertebrates. Individual-level lateralisation, wherein individuals within the population vary in their preference, has been documented in the desert locust (*Schistocerca gregaria*), which swarms but is not considered social. The red wood ant (*Formica rufa*) lives in social groups providing an opportunity to study the possible effects of social factors on the evolution of handedness in insects. Whilst crossing a gap in the substrate upon which they are walking, ants use their forelimbs to reach across and contact the opposite side. We investigated whether they displayed a preference for using a particular forelimb. In this context, some individual ants preferred to use their right forelimb, others their left and the remainder showing no preference – the hallmark of individual-level handedness. Remarkably, the preference differed between colonies, the majority of individuals within a colony showing a preference for using the same forelimb to cross the gap. Thus, wood ants exhibit two forms of handedness at the individual and colony-levels the latter being an entirely novel, previously undescribed form of lateralisaton.

A.1 Ant colonies display a directional bias in limb preference

Adapted, with permission, from Section 3.4.6 of A. Bell (submitted). 'On the lateralized motor behaviour of insects'. PhD thesis. University of Sussex.

Were colonies of ants unbiased, observations of their forelimb preference should approximate a binomial distribution. Instead, the distribution we observed deviated significantly from the binomial expectation in all four colonies (Colony 1: G-test, $G_{adj} = 17.65, 2df, p < 0.005, N = 50$; Colony 2: G-test, $G_{adj} = 14.06, 3df, p < 0.005, N = 50$; Colony 3: G-test, $G_{adj} = 20.12, 3df, p < 0.005, N = 50$; Colony 4: G-test, $G_{adj} = 12.61, 3df, p < 0.01, N = 46$) indicating colonies of ants display a forelimb preference.

If colonies of ants were to exclusively display individual-level lateralisation the number of observations would be split evenly within the colony between left and right handed individuals. Instead, the distributions are shifted towards a particular direction with the majority of individuals sharing the same preference, indicating a bias in the colonies.

We find Colony 1's distribution is weighted towards the left, with a mean proportion of right forelimb reaches of 0.41 (median = 40), indicating a preference for the left forelimb. In comparison, we find the other three colonies distributions shifted towards the right, with colony Colony 2 a mean proportion of right forelimb reaches of 0.56 (median = 60), Colony 3 a mean proportion of right forelimb reaches of 0.59 (median = 58.6) and Colony 4 a mean proportion of right forelimb reaches of 0.58 (median = 62.5), indicating a colony-level bias for the right forelimb.

These results indicate red wood ants display a preference for using a particular forelimb whilst gap crossing. In this context, some individual ants preferred to use their right forelimb, others their left and the remainder showed no preference – the hallmark of individual-level lateralisation. Remarkably, the preference differed between colonies, the majority of individuals within a colony showing a preference for using the same forelimb to cross the gap. Thus, wood ants exhibit two forms of lateralisation at the individual-level and at the colony-level. This latter form of lateralisation is entirely novel and previously undescribed.

Colony	**Number of ants tested**	**Trials per ant**	**Mean proportion of right forelimb reaches**	**Index of laterality**
1	50	10	0.4080	-0.1840
2	50	10	0.5640	0.1280
3	50	10	0.5860	0.1720
4	46	8	0.5788	0.1576

Table A.1: Colony-level lateralization in four colonies of *Formica rufa*. Data gathered by Adrian Bell, with Jamie Sneddon and Johnathan Stone.

Appendix B

An environmental model of self-compatibility transitions in the Solanaceae plant family

P. Calcraft et al. (2013). 'An environmental model of self-compatibility transitions in the Solanaceae plant family'. In: *Advances in Artificial Life, ECAL*. vol. 12. Cambridge MA, USA: MIT Press, pp. 1107–1113.

An Environmental Model of Self-Compatibility Transitions in the Solanaceae Plant Family

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Abstract

Higher level selection processes such as species selection are not generally predicted to overpower individual selection on character traits. Goldberg et al. provide a model derived from collected life history data and argue that species selection is maintaining self-incompatibility in the Solanaceae plant family. This model applies only on the level of the species, not representing the underlying interactions between individuals and the environment. We propose a new model with environmental variation at the individual level that may explain the maintenance and frequency of loss of this character trait. We use individual based modelling techniques to explore our hypothesis, and compare it with that originally proposed. The results show alternative values required for the mutation rate to produce the species-level transition frequency under the opposing models, given certain assumptions. Future work is suggested to refine the parameter relationships, test for robustness, and determine if individual models of higher complexity will exhibit similar outcomes.

Introduction

Evolutionary questions that address multiple levels of the biological hierarchy offer a particular challenge to researchers. There is lack of consensus among biologists about the level(s) at which Darwinian natural selection should be considered to act (Okasha, 2006). This debate about the levels of selection has a complex history, marked by the group selection controversy (Wilson, 1983; Okasha, 2001), and theories of multi-level selection (Damuth and Heisler, 1988) and inclusive fitness (Hamilton, 1964; Queller, 1992).

Empirical data concerning the life history of individuals and species is in many cases insufficient to answer biological questions conclusively (Johnson and Omland, 2004; Turchin, 2013). Mathematical models have been used extensively, but they cannot capture the complexity of interactions in all cases. Individual based modelling techniques can be used to approach problems of this nature, using computer simulations of interacting systems at multiple levels. These models can expose outcomes of theoretical positions that may not be readily apparent (Di Paolo et al., 2000). Their flexibility and speed additionally allow systematic exploration of parameter spaces, testing the robustness and plausibility of proposed ideas. Their potential for incorporating environmental interaction can be key in exposing the workings of natural systems (Brooks, 1991).

In this paper we model the individual interactions within the species that underlie the model of Goldberg et al. (2010). We consider individual level selection, that is, natural selection competing between individuals within a given species, to try to expose the lower level dynamics that are giving rise to the outcomes seen at the level of species. We compare two alternative formulations with different profiles of environmental interaction; the first assuming that, as proposed by Goldberg et al., species selection is acting in direct opposition to the lower level individual selection; the second introducing environmental variation. Individual level selection processes are generally considered more powerful than their higher level counterparts such as species selection (Lewontin, 1970). Species selection is therefore rarely cited as able to maintain a trait that is disadvantageous to the individual, but this has been suggested in the case of self-incompatibility in the Solanaceae (nightshade) plant family (Goldberg et al., 2010).

In the next section we discuss the evolutionary background of self-incompatibility in the context of Goldberg et al.'s work. We then introduce the theoretical concepts behind the competing evolutionary incentives. Following this, we lay out the mathematical model of fitness that will form the basis of our individual model. With the mathematical framework in place, we describe the details of the computer simulation and the relevant parameters. Finally, we go on to discuss the results.

Self-Incompatibility and the Goldberg et al. Model

Self-incompatibility (SI) in plants is a mechanism to prevent self-fertilisation and encourage outcrossing – reproduction with those genetically dissimilar; this increases the genetic diversity of offspring (Barrett, 1988). The alternative, however – self-compatibility (SC) – can be immediately evolutionarily advantageous to individuals in SI populations, as self-fertilising (or selfing) allows plants to pass on their

genes with higher probability, and selfing plants need not rely on inbound pollen when it is scarce (Lloyd, 1992; Igic et al., 2008).

Goldberg et al. reconstructed a tree of life for the nightshade family, and found a maximum likelihood model that captures the evolution of the species in the family and their relationship with self-incompatibility and self-compatibility. The model shows that SI species have an average rate of transition to SC of 0.555 per lineage per million years, yet a proportion of SI species continues to survive over evolutionary time. This appears to be because SI species have a higher net rate of growth than SC species. This difference (a species-level advantage) is greater than the rate of transition, allowing a proportion of species to be maintained as SI ongoing, despite regular transition to SC.

In claiming that species selection is maintaining self-incompatibility, Goldberg et al. argue that individual evolutionary incentive for SC is constantly present, but the rate of arrival (and spreading) of the SC mutation in each SI population is sufficiently low to keep the transition rate averaging at this 0.555 per million years figure. This rate is low enough to allow the difference in net species growth (diversification) to be the more significant evolutionary force.

We propose a model where a background factor, a rate of occurrence of temporary environmental disruption, is the cause for a given species transition to SC. Under this model, individual selection does not constantly favour selfing, but is rather selectively neutral, or marginally favouring outcrossing. When the environmental disruption occurs, the relative fitness of selfers increases temporarily, and there is opportunity for an SC mutant to arrive and spread in the population. As long as this model can be shown to achieve the same average transition rate for reasonable sets of parameters, it may offer an alternative explanation for the maintenance of outcrossing that does not require species selection to overpower individual selection unaided.

It is important to note that while transitions from SI to SC are regular and frequent, transitions back to SI are negligibly rare (Igic et al., 2008). This may be due to the complexity of the SI system; it requires genetic coordination at many loci, so there are many points of failure (Franklin-Tong, 2008). There may also be a self-perpetuating dynamic to selfer evolution, as under certain conditions, an increase in the proportion of selfers also increases selfer fitness, making evolution back to SI once a species has been fully invaded by SC particularly improbable.

Individual Selection Models of Selfing versus Outcrossing

A strong individual incentive for selfing is believed to be its transmission advantage, termed *automatic selection* (Fisher, 1941). Selfers have a 3:2 advantage of gene transmission, as their seeds on average contribute two gametes to the next generation to the outcrosser's one (while both averaging an additional one through pollen) (Busch and Delph, 2012). This transmission advantage is opposed by inbreeding depression – a generalised concept for the lower average fitness of selfed progeny. Selfed progeny may have lower fitness for a number of reasons, including reduced genetic diversity, and exposition of harmful recessive alleles (Charlesworth and Charlesworth, 1987). In simple models, inbreeding depression is represented by a value δ which is the per progeny reduced fitness for a selfed individual. If $\delta > 0.5$, the selfer's transmission advantage is outweighed by inbreeding depression, and outcrossing is evolutionarily preferred (Jarne and Charlesworth, 1993): 0.5 is the equilibrium level of inbreeding depression in this model of transmission advantage. This simple relation assumes that selfer pollen is just as successful as outcrosser pollen, ignoring any pollen discounting. Pollen discounting, $0 \leq p \leq 1$, is the reduced relative fitness of pollen spores for selfer pollen (Nagylaki, 1976).

Using Lloyd (1992)'s phenotypic model of selfing versus outcrossing, a non-zero level of pollen discounting results in a frequency dependent equilibrium value for inbreeding depression δ (Nagylaki, 1976). That is, the maximum level of inbreeding depression required to prevent evolution to selfing varies with the proportion of selfers (explained below with eq. (3)). It can therefore provide a self-perpetuating dynamic to the evolution of selfing, as the level of δ required to maintain outcrossing increases with the proportion of selfers, so as more selfers evolve, it becomes increasingly more difficult to maintain outcrossing. This means that if environmental circumstances are temporarily in a state that encourages evolution to SC, the proportion of outcrossers may drop below the level at which outcrossing could be maintained even once the environment returns to its previous state. This is a mechanism by which, without any assumed change in inbreeding depression, a temporary environmental disruption may cause one-way transitions to SC.

Our proposed environmental disruption is a temporary limitation of pollen dispersal in the population. This reduces inbound pollen availability to outcrossing plants by an amount $0 \leq l \leq 1$. This limitation also has fitness consequences for selfers, as outbound pollen from selfer plants will be less likely to reach and sire an outcrosser ovule for reproduction. The limitation still has a greater negative effect on outcrossers than selfers, as self-fertilised seeds will be unaffected by the lack of pollen dispersal, while all outcross progeny will be penalised.

The Mathematical Model of Selfing versus Outcrossing

For general pollen limitation l, the initial fitness of outcrossers W_x and selfers W_s are:

$$W_x = 1 - l \tag{1a}$$

$$W_s = 1 - \delta \tag{1b}$$

This corresponds to inbreeding depression for selfers, and inbound pollen limitation for outcrossers. The transmission advantage also needs to be factored in, for which we adapt the model from (Lloyd, 1992). The transmission advantage is the result of an additional crossover process for outcrossers, in which their offspring have an average 50% chance of transmission of the trait carried by the inbound pollen (rather than their own) (Fisher, 1941). The outcrosser fitness is therefore scaled by $\frac{1}{2} + \frac{1}{2}m_x$, where m_x is the probability the mate is also an outcrosser. The complement of this amount ($\frac{1}{2} - \frac{1}{2}m_x$) is added to the selfer fitness (representing those outcrosser progeny transmitting the selfer phenotype). This term, however, is scaled by the relative proportion of outcrossers to selfers and reduced by pollen limitation, as selfers only benefit as much as there are outcrosser ovules available to sire and their pollen can reach them. The comprehensive fitness equations are therefore:

$$W_x = (1-l)\frac{1}{2}(1+m_x) \tag{2a}$$

$$W_s = \frac{x}{1-x}(1-l)\frac{1}{2}(1-m_x) + 1 - \delta \tag{2b}$$

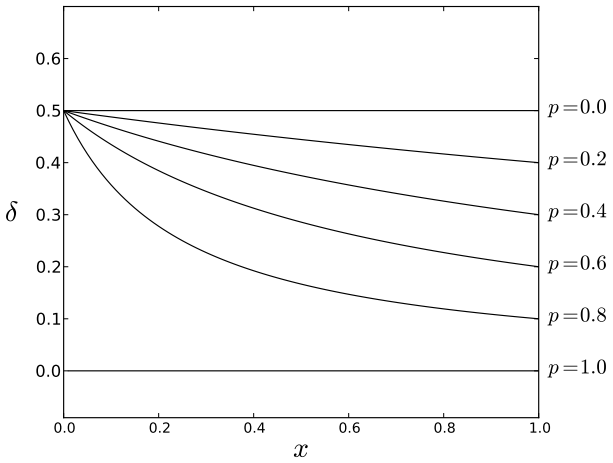
$$\text{where} \quad m_x = \frac{x}{x+(1-x)(1-p)} \tag{2c}$$

Again, m_x is the probability of inbound pollen being outcrosser rather than selfer, incorporating the effect of pollen discounting p. The current proportion of outcrossers in the population is $0 \leq x \leq 1$.

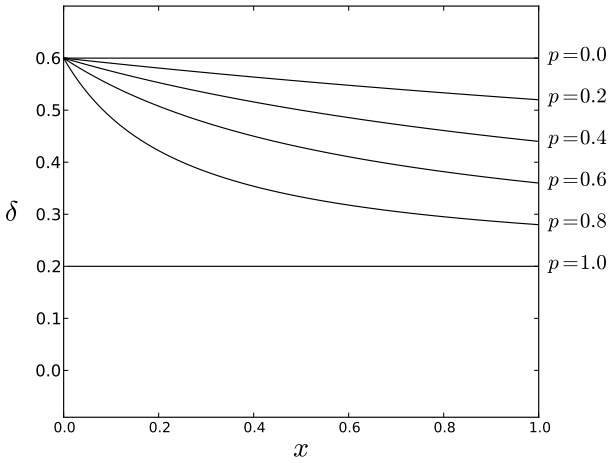
From these fitness equations we derive the level of equilibrium inbreeding depression δ, above which outcrossing is evolutionarily preferred, and below which selfing is preferred:

$$\frac{2lpx + (1+l)(1-p)}{2(p(x-1)+1)} \tag{3}$$

Refer to figs. 1(a) and 1(b) for an illustration of this relationship. As we can see, for $0 < p < 1$, the level of inbreeding depression required to maintain outcrossing increases with selfer proportion (ie. with decreasing x); selfers have a greater advantage as the selfer proportion increases. Further, the addition of pollen limitation $l = 0.2$ in fig. 1(b) shifts the curves upward, giving selfers a selective advantage over the $l = 0$ condition. The curves are also contracted in the vertical (δ) dimension, making this difference more pronounced at higher levels of p. We use changes in the value of l to exhibit temporary environmental conditions that favour selfing.



(a) Equilibrium δ without pollen limitation ($l = 0$)



(b) Equilibrium δ under pollen limitation ($l = 0.2$)

Figure 1: Equilibrium inbreeding depression δ at outcrosser proportion x for different values of pollen discounting p, with (1(b)) and without (1(a)) pollen limitation l (see eq. (3)).

We present two alternative models. In the first, Model A, the transition rate is caused exclusively by the arrival and fixation of the selfer mutation, under conditions that constantly favour selfing. In Model B, conditions generally favour outcrossing, but there are environmental disruptions, occurring with a certain rate r, that limit l the pollen dispersal for some duration d, during which the conditions favour selfing. This second model, the environmental model, will require a higher mutation rate than the first, as selfer morphs are only favoured by natural selection during disruptions, rather than constantly. The final point of differentiation for the two models, then, will be the mutation rate required to

achieve the empirically observed transition rate, given the background assumptions of the models.

Methods

As stated, the target is an average transition rate of 0.555/million years. We run many repeats (500) of a single population under both conditions (original Model A and environmental disruption Model B), recording the number of years it takes to transition to selfing each time. We then take the reciprocal of the mean length of time, arriving at the average transition frequency. After fixing certain parameters of the models, we search manually for parameters that produce the target transition rate for these conditions. Using the same criterion as Goldberg et al. (2010), we conservatively classify a species as SI as long as it is not completely SC, ie. no polymorphism, approximated as less than 1% of the SI phenotype present in the population; a transition is said to occur when the outcrosser proportion goes below 0.01.

The simulation is a genetic algorithm with a single population, initially fully outcrossing ($x = 1$). We use roulette selection, which is equivalent to a diffusion approximation of selection and drift (Cherry and Wakeley, 2003). The fitness values for outcrossers and selfers are as per eqs. (1a) and (1b).

Upon selection, if the phenotype is selfer, it is added to the next generation, but if outcrosser, it is combined with pollen from another plant in the population. The probability that this mate is an outcrosser, as opposed to selfer, is m_x (eq. (2c)).

The phenotype that goes into the next generation is from either the selected plant or the mate, with equal probability. This is equivalent to the average effect of crossover for outcrossing plants. The net effect of this selection and probabilistic recombination process is captured in fitness eqs. (2a) and (2b). The trait is also probabilistically mutated according to the (phenotypic, per gene per generation) mutation rate μ before being added to the next generation.

After each generation, we check if the population has transitioned to SC ($x < 0.01$) and break out of the current run if this is the case, recording the length of time that has passed. One generation is equal to one year, a working value used by other models of plants in the Solanaceae family (Vekemans and Slatkin, 1994). For a high level overview of the computer simulation's operation, refer to algorithm 1.

```
1  for each repeat do
2      generations_until_transition = 0;
3      while outcrosser_proportion > 0.01 do
4          if disruption_generations_remaining = 0 then
5              pollen_limitation = 0;
6          else
7              disruption_generations_remaining -= 1;
8          end
9          if random() < disruption_rate {r} then
10             pollen_limitation =
                 disrupted_pollen_limitation {l_d};
11             disruption_generations_remaining =
                 disruption_length {d};
12         end
13         for population size {N_e} do
14             roulette select an individual;
15             if individual is outcrosser then
16                 pick mate according to pollen
                     frequencies {m_x};
17                 crossover with mate;
18             end
19             probabilistically mutate {μ};
20             add to new generation;
21         end
22         generations_until_transition++;
23     end
24     record generations_until_transition;
25 end
26 print 1/(average(generations_until_transition));
```

Algorithm 1: Model algorithm

Parameters

Table 1 shows the initial set of parameters for the models. The effective population size N_e for Solanaceae does vary, but 6000 is within the expected range (Richman et al., 1996). A conservative level of pollen discounting, 0.2, has been chosen initially. As explained, Model A requires that the conditions favour selfing constantly, so a value of $\delta = 0.3$ has been chosen to fulfil this requirement (see fig. 1(a), 0.3 is below the δ equilibrium for $p = 0.2, l = 0$). For Model B, the environmental disruption model, we need selective neutrality or favoured outcrossing under background pollen limitation $l = 0$ (fig. 1(a), 0.5 is above the δ equilibrium for $p = 0.2$, $l = 0$), and favoured selfing under the disruption condition (fig. 1(b), 0.5 is below the δ equilibrium for $p = 0.2, l = l_d = 0.2$).

	Parameter description	Model A	Model B
p	Pollen discounting rate	0.2	
l	Background pollen limitation	0	
l_d	Pollen limitation (disrupted)	N/A	0.2
N_e	Effective population size	6000	
δ	Inbreeding depression	0.3	0.5
μ	Mutation rate	*	*
r	Disruption rate, /species/generation	0	*
d	Disruption duration, generations	0	*

Table 1: Parameters of the model under Models A and B. Values to be found or manipulated are marked by *.

Results

In the first section we present the results from Model A, the model under which species selection directly opposes constant individual incentive for selfing, and Model B, where environmental disruptions bring about temporary individual incentive for selfing. We indicate parameter values under which these alternative low level models exhibit the empirically observed transition rate of 0.555 per lineage per million years (0.555E-6) at the species level. We then go on to present some typical evolutionary trials, exposing the underlying selection mechanics of the models.

Results from Models A and B

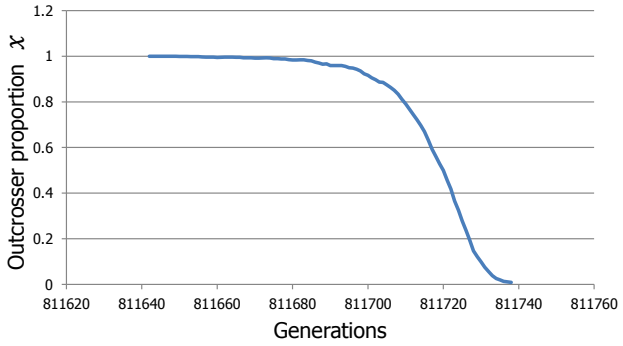
In each case, the resultant transition rate is the mean frequency of transition over 500 trials of the single population genetic algorithm. Parameter values were found by manual experimentation given the fixed values established by the model assumptions, detailed previously in table 1. The output parameter of interest is the phenotypic mutation rate μ required under each model to bring about the rate of transition observed by Goldberg et al..

	μ	r	d	Transition rate
A	5.17E-10	0	0	0.547E-6
$\mathbf{B}_0$	1.33E-8	⟨1E-5⟩	5000	0.563E-6
$\mathbf{B}_1$	2.17E-8	⟨1E-5⟩	3000	0.537E-6
$\mathbf{B}_2$	2.17E-7	⟨1E-5⟩	500	0.552E-6
$\mathbf{B}_1$	2.17E-8	1E-5	⟨3000⟩	0.537E-6
$\mathbf{B}_3$	2.28E-8	5E-6	⟨3000⟩	0.567E-6
$\mathbf{B}_4$	2.33E-8	1E-6	⟨3000⟩	0.572E-6

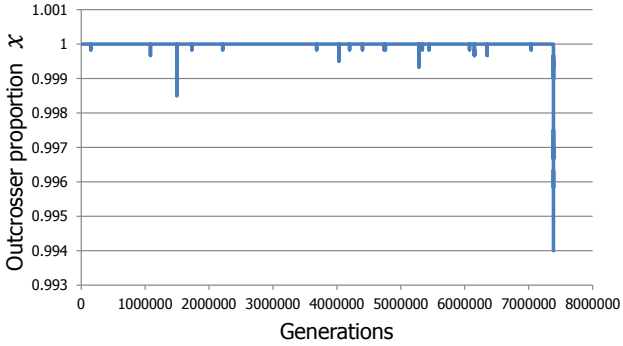
Table 2: Parameters and results under Model **A** (original, no disruption: $r, d = 0$) and **B** (temporary environmental disruptions: $r, d > 0$). Transition rate should approximate 0.555E-6. The table is grouped, where values held constant are shown in angle brackets, while others were manipulated to obtain the target transition rate. Result B_1 is repeated in the third group for convenient comparison.

Table 2 shows that a transition rate of approximately 0.555E-6 can be obtained under multiple conditions; either model is able to potentially explain the empirical observations, but with a different necessary value for the mutation rate μ. For Model A, the background assumptions are such that there is only one possible value, found to be 5.17E-10. Under Model B there is more scope for interaction between the parameters during the search. Holding the disruption rate r at an average of once per 100,000 years (1E-5), rows B_0, B_1, and B_2 show that higher mutation rates are required for shorter durations of disruption. Keeping the disruption duration d at 3000 years, we similarly see from rows B_1, B_3, and B_4 that lower values of disruption rate r require higher mutation rates, but the effect is considerably less significant. The required mutation rate is more sensitive to the duration of the disruptions than their frequency.

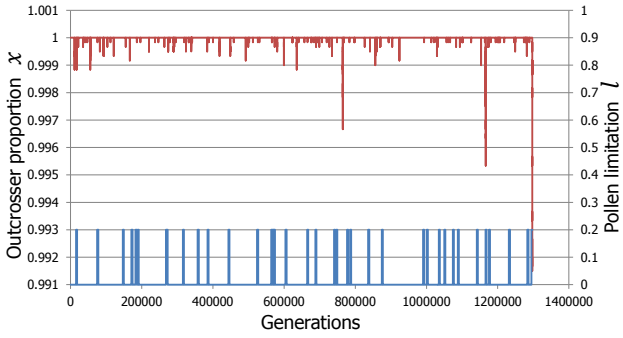
Example Evolutionary Trials



(a) A final phase of the evolution curve once selfing manages to spread, typical under both models.



(b) An example evolution curve under Model A where SC mutations arise and are lost multiple times under drift before managing to spread and fixate. The final line down on the right continues to full selfing as in fig. 2(a). Note the scale of the y axis: x does not get below 0.9985 without SC spreading.



(c) An example evolution curve under Model B. The level of pollen limitation, alternating between 0 and 0.2 on the secondary y axis due to disruption, is also shown. Observing the scale of the primary y axis, x (the top curve) reaches below 0.9955 without SC managing to spread, lower than under Model A in fig. 2(b). The final line down on the right again continues to full selfing as in fig. 2(a).

Figure 2: Example sequences from typical evolutionary runs.

Under Model B, the temporary disruptions in the environment allow SC mutations to arise and begin to spread if they are not lost by drift, and may get further than is typical under Model A. This is illustrated in figs. 2(b) and 2(c), where the different scales of the y axis show that the outcrosser proportion can typically get slightly lower in B without a full SC invasion. This is likely due to the inconsistent selection pressure provided by Model B, as disruptions are brought in and out, shifting the balance of fitness towards and away from outcrossing over time.

Both models produce similar final phases of SC spreading to fixation, as seen in fig. 2(a), as under either model, once selfers reach a certain proportion, selection pressure becomes reinforcing and full invasion becomes highly probable.

Discussion

Evolutionary models that consider the interaction between multiple levels of the biological hierarchy provide a complex challenge. We have taken Goldberg et al.'s species-level empirical data and attempted to realise the individual level processes that give rise to the SI-to-SC species transition rate. Using a genetic algorithm and Lloyd (1992)'s phenotypic model, we discover the mutation rates required under two alternative models, given certain assumptions.

We begin to explore the conditions under which the target transition rate can be produced, and show that there seems to be scope for an environmental model to help explain the evolutionary history of SI and SC in the Solanaceae plant family. Assessing the likelihood of the presented model, or of alternative environmental variation hypotheses, will come down to the plausibility of the required mutation rates. If the mutation rate required of Model A, under the pure species selection hypothesis, is too low, this may suggest individual selection is a significant factor, mediated by environmental conditions. Our Model B presents one such possibility.

The method presented of separating out the individual selection process from the species-level process may be applicable to other questions regarding multi-level selection processes. By starting with empirical evidence at the species level and reverse engineering the individual selection pressure using established models, we can explore the real world parameter ranges required to meet alternative theories. These parameters can then hopefully be subject to empirical test, to observe which model obtains. It should however be noted that this method has its limitations: while the two levels of selection can be isolated relatively cleanly, there is some scope for interaction effects, and a more comprehensive model that includes both levels of selection would be a logical extension to this work.

We did not have time to perform more comprehensive parameter sweeps to provide a robustness analysis. Investigating the relationships between the sets of parameters may prove fruitful as well.

In addition to testing for interaction effects by extending the model to multiple simultaneous levels of selection, future work should also explore alternative theories of environmental variation. In the first instance, an alternative take on Model B would be to have pollen limitation l vary continuously in the background, rather than being manipulated by binary disruption events. It may be that gradual or shallower yet longer dips in dispersal can produce similar rates of transition, for example. Another valuable extension would be the incorporation of more complex models of inbreeding depression and pollen discounting, as unforeseen interactions between environmental variation and fitness over time may be exhibited.

In summary, we have examined within-species dynamics, under individual selection, that can account for the species-level rate of transition that has been empirically observed. Given certain conditions, we obtained the values necessary for the mutation rate to explain the data under two alternative models. Individual based modelling techniques were effectively employed, enabling the analysis of these stochastic models under environmental interaction. By attempting to establish the details of the biological interactions below the species level, we indicate parameter values that may support or reject the original species selection hypothesis.

References

Barrett, S. C. (1988). The evolution, maintenance, and loss of self-incompatibility systems. *Plant reproductive ecology: patterns and strategies*, pages 98–124.

Brooks, R. A. (1991). Intelligence without representation. *Artificial intelligence*, 47(1):139–159.

Busch, J. and Delph, L. (2012). The relative importance of reproductive assurance and automatic selection as hypotheses for the evolution of self-fertilization. *Annals of botany*, 109(3):553–562.

Caballero, A. et al. (1994). Developments in the prediction of effective population size. *Heredity*, 73(6):657–679.

Charlesworth, D. and Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual review of ecology and systematics*, 18:237–268.

Cherry, J. L. and Wakeley, J. (2003). A diffusion approximation for selection and drift in a subdivided population. *Genetics*, 163(1):421–428.

Damuth, J. and Heisler, I. L. (1988). Alternative formulations of multilevel selection. *Biology and Philosophy*, 3(4):407–430.

Di Paolo, E. A., Noble, J., and Bullock, S. (2000). Simulation models as opaque thought experiments.

Fisher, R. (1941). Average excess and average effect of a gene substitution. *Annals of Human Genetics*, 11(1):53–63.

Franklin-Tong, V. E. (2008). *Self-incompatibility in flowering plants: evolution, diversity, and mechanisms*. Springer.

Goldberg, E. E., Kohn, J. R., Lande, R., Robertson, K. A., Smith, S. A., and Igić, B. (2010). Species selection maintains self-incompatibility. *Science*, 330(6003):493–495.

Hamilton, W. D. (1964). The genetical evolution of social behaviour. i. *Journal of theoretical biology*, 7(1):1–16.

Holsinger, K. (1991). Mass-action models of plant mating systems: the evolutionary stability of mixed mating systems. *American Naturalist*, pages 606–622.

Holsinger, K. (1996). Pollination biology and the evolution of mating systems in flowering plants. *Evolutionary biology*, 29.

Igic, B., Lande, R., and Kohn, J. R. (2008). Loss of self-incompatibility and its evolutionary consequences. *International Journal of Plant Sciences*, 169(1):93–104.

Jarne, P. and Charlesworth, D. (1993). The evolution of the selfing rate in functionally hermaphrodite plants and animals. *Annual Review of Ecology and Systematics*, pages 441–466.

Johnson, J. B. and Omland, K. S. (2004). Model selection in ecology and evolution. *Trends in ecology & evolution*, 19(2):101–108.

Lande, R. and Schemske, D. W. (1985). The evolution of self-fertilization and inbreeding depression in plants. i. genetic models. *Evolution*, pages 24–40.

Lewontin, R. C. (1970). The units of selection. *Annual Review of Ecology and Systematics*, 1:1–18.

Lloyd, D. G. (1992). Self-and cross-fertilization in plants. ii. the selection of self-fertilization. *International Journal of Plant Sciences*, pages 370–380.

Nagylaki, T. (1976). A model for the evolution of self-fertilization and vegetative reproduction. *Journal of Theoretical Biology*, 58(1):55–58.

Okasha, S. (2001). Why won't the group selection controversy go away? *The British journal for the philosophy of science*, 52(1):25–50.

Okasha, S. (2006). *Evolution and the Levels of Selection*, volume 16. Clarendon Press Oxford.

Queller, D. C. (1992). Quantitative genetics, inclusive fitness, and group selection. *American Naturalist*, pages 540–558.

Richman, A. D., Uyenoyama, M. K., and Kohn, J. R. (1996). Allelic diversity and gene genealogy at the self-incompatibility locus in the solanaceae. *Science*, 273(5279):1212–1216.

Turchin, P. (2013). *Complex population dynamics: a theoretical/empirical synthesis (MPB-35)*, volume 35. Princeton University Press.

Vekemans, X. and Slatkin, M. (1994). Gene and allelic genealogies at a gametophytic self-incompatibility locus. *Genetics*, 137(4):1157–1165.

Wilson, D. S. (1983). The group selection controversy: history and current status. *Annual review of ecology and systematics*, 14:159–187.